RELEASE OF MICROORGANISMS FROM SOLID MATERIALS

Prepared for

CALIFORNIA INSTITUTE OF TECHNOLOGY
JET PROPULSION LABORATORY
CONTRACT NUMBER 952916

SEP 1971
RECEIVED
NASA STI FACILITY
INPUT BRANCH

Prepared by

E. A. Gustan

R. L. Olson, Ph. D.

THE BOEING COMPANY
Aerospace Group
Seattle, Washington

FACILITY FORM 602

N71-34056
(ACCESSION NUMBER)

86
(PAGES)

CR-121707
(NASA CR OR TMX OR AD NUMBER)

(THRU)

G3
(CODE)

04
(CATEGORY)

Reproduced by
NATIONAL TECHNICAL INFORMATION SERVICE
Springfield, Va. 22151

RELEASE OF MICROORGANISMS FROM SOLID MATERIALS

F I N A L   R E P O R T

July 1971

Prepared for

CALIFORNIA INSTITUTE OF TECHNOLOGY
JET PROPULSION LABORATORY
CONTRACT NUMBER 952916

"This work was performed for the Jet Propulsion Laboratory, California Institute of Technology, as sponsored by the National Aeronautics and Space Administration under Contract NAS 7-100."

Prepared by

E. A. Gustan
R. L. Olson, Ph. D.

THE BOEING COMPANY
Aerospace Group
Seattle, Washington

ABSTRACT

RELEASE OF MICROORGANISMS FROM SOLID MATERIALS

This contract consisted of three study phases that provided information on the release of microorganisms by hard impact and determined the effect of aeolian erosion on the release of microorganisms. The first phase was initiated to determine the efficiency of grinding, as compared to dissolution, for recovery of microorganisms from solids. An adjustment constant of 20 was derived from the data that can be used to equate bacterial spore counts obtained by grinding with those obtained by dissolution.

Phase II was conducted to determine the percentage of microorganisms released due to hard impact of Eccobond onto sand. This study provides additional data to JPL Contract Number 952511. In this study, Eccobond was impacted onto sand at velocities of 168, 457, 945 and 1554 m/sec. The results showed that less than 1 percent of the available organisms was released by impact.

The Phase III study was initiated to determine the percentage of bacterial spores released from methyl methacrylate and Eccobond by aeolian erosion. Sand, accelerated by air or carbon dioxide, was used to erode 0.25 grams of material from one gram discs. The results showed that less than 1 percent of the available organisms was released by the erosion process.

TABLE OF CONTENTS

SECTION I

SECTION I

SUMMARY

RELEASE OF MICROORGANISMS FROM SOLID MATERIALS

SUMMARY

RELEASE OF MICROORGANISMS FROM SOLID MATERIALS

This is the final report submitted by The Boeing Company to the Jet Propulsion Laboratory on JPL Contract Number 952916. Three research phases were conducted for the contract that extended from July 1970 through June 1971. These phases are discussed separately in this report in Sections II, III and IV respectively. A summary of the total contract effort is presented in Section I.

INTRODUCTION

Viable terrestrial microorganisms may be trapped in certain solid spacecraft materials and survive decontamination or terminal sterilization processes. These surviving organisms are then available for release into planetary environments by fracturing of the solid materials due to a hard impact of the vehicle and by environmental erosion. Data are required on these release mechanisms in order to evaluate the probability of microbial release from spacecraft within the planetary contamination constraints established by the Committee on Space Research (COSPAR) and the National Aeronautics and Space Administration (NASA). The probability of release evaluations can have a significant impact on the spacecraft terminal sterilization cycle as well as the flight acceptance cycles by their inclusion in a total probabilistic systems analysis of contaminating events. The objective of this investigation was to obtain information that can be used in probability calculations related to planetary quarantine.

From a previous study, "Release of Microorganisms From Solids After Simulated Hard Landings," (JPL Contract No. 952511), additional research areas were identified which make up the three phases for this investigation. Phase I was conducted to determine the efficiency of a grinding technique to release microorganisms for enumeration from the interior of a solid material. Data was obtained for purposes of calculating an adjustment constant to be used in adjusting the numerical counts obtained by the

grinding technique. The second phase was conducted to determine the number of bacterial spores released from inoculated Eccobond after a hard impact onto sand. This release data provides additional information to supplement the hard impact release studies conducted previously. Phase III was conducted to provide information on the effect of aeolian erosion on the release of microorganisms from solid materials. The data obtained can be used to determine the probability of microbial release due to this erosion process.

PHASE I

GRINDING EFFICIENCY STUDIES FOR MICROBIAL RECOVERY

A series of tests were initiated in which dissolving and grinding techniques were employed to recover bacterial spores from methyl methacrylate. Spore recovery data was also obtained from Eccobond by grinding. The data generated during these tests were used to calculate an adjustment constant that is used to equate the microbial counts obtained by grinding to the counts obtained by dissolution. The spore counts obtained by grinding are multiplied by the adjustment constant which was calculated to be 20.

In addition, tests were conducted on the size of particles resulting from grinding methyl methacrylate and Eccobond and the number of spores associated with these particles. The data indicates that the ground Eccobond particles appeared somewhat smaller than the methyl methacrylate particles. However, the number of spores that grew on the surfaces of both groups of particles was approximately the same. This discrepancy may be due to the sieving method that was used for sizing the particles since it was not possible to determine the exposed surface area directly.

PHASE II

IMPACTION OF INOCULATED ECCOBOND ONTO SAND

Tests were conducted to determine the number of bacterial spores released from internally inoculated Eccobond pellets after a hard impact onto sand. One gram Eccobond pellets were fabricated so as to contain

approximately 1×10^4 *Bacillus subtilis* var. *niger* spores. These pellets were propelled from a gun at 168, 457, 945 and 1554 meters per second into sand. After impact, the pellet fragments and sand were assayed to determine the number of viable spores released.

The data showed that less than 1 percent of the available organisms in the pellets was released upon impact at the four test velocities. These results are consistent with the results from previous hard impact studies.

PHASE III

EROSION OF INOCULATED METHYL METHACRYLATE AND ECCOBOND BY SAND BLASTING

A series of tests were conducted to determine the effect of the erosive action of sand on the release of bacterial spores from solid materials. Internally inoculated methyl methacrylate and Eccobond 1 gram discs were subjected to simulated aeolian erosion in a specially designed sand blasting apparatus. Approximately 0.25 grams were eroded from each disc for each of 4 exposure conditions; 0.5, 2 and 24 hours using air and 0.5 hours using carbon dioxide. After each erosion cycle, the contents of the sand blasting apparatus were assayed for released viable spores.

The results showed that less than 1 percent of the available spores was released for all test conditions. An analysis of variance of the percent of spores released showed no significant differences between the test conditions or between the 2 materials.

SECTION II

SECTION II

PHASE I

GRINDING EFFICIENCY STUDIES FOR MICROBIAL RECOVERY

TABLE OF CONTENTS

PHASE I

GRINDING EFFICIENCY STUDIES FOR MICROBIAL RECOVERY

1.0 PURPOSE

This phase of the program was conducted to determine the efficiency of a grinding technique used to recover bacterial spores for enumeration from internally inoculated materials.

2.0 INTRODUCTION

Results from a previous investigation (JPL Contract No. 952511, "Release of Microorganisms From Solids After Simulated Hard Impact") indicated that when Eccobond was impacted onto stainless steel, the percentage of spores released was approximately ten times greater than the number of spores released from methyl methacrylate under the same conditions. This difference could be attributed to a material effect or to differences in assay procedures, i.e., Eccobond was ground to obtain the initial number of organisms present in pellets before firing while methyl methacrylate was dissolved. A gross visual examination of the fractured Eccobond and methyl methacrylate pellets showed generally similar characteristics with respect to fracture patterns, surface areas and particle sizes after impact. The minor differences that were observed in fracturing did not appear great enough to account for the differences observed in the percentage of spores released in the two materials. It was, therefore, concluded that the assay techniques and material fabrication should be investigated. This study was initiated to investigate these observed differences and to determine the efficiency of grinding materials for microbial enumeration.

3.0 PROCEDURE

Methyl methacrylate and Eccobond pellets were inoculated and fabricated so that each pellet contained approximately 5×10^4 *Bacillus subtilis* var. *niger* spores per gram of finished material. Recovery of the spores was obtained from methyl methacrylate by dissolution and grinding techniques and from Eccobond by grinding only. Information was also obtained from methyl methacrylate and Eccobond on the size of the particles resulting

from the grinding process. The data from these parallel studies are used to calculate an efficiency factor for the grinding process. The steps and sequence used for the parallel studies are outlined in Figure 1.

3.1 PELLET MANUFACTURE

3.1.1 Preparation of Spore Stock

A 0.1 ml aqueous suspension containing 10^8 *B. subtilis* spores was placed in the bottom of sterile planchets. The stock spore suspension was a minimum of 6 months old. The planchets were dried overnight by airflow from a Class 100 clean bench. When all the water had evaporated, 10 planchets were placed in a bottle containing 30 ml of ethanol and insonated for 20 minutes. The level of the bath water was adjusted so that it was halfway up the side of the bottle. After insonation, the 30 ml spore-ethyl alcohol suspension was pipetted into a sterile, capped bottle. All planchets were rinsed in fresh ethanol and the washings plus additional ethanol was combined with the 30 ml stock to bring the total to 100 ml. The stock inoculum was refrigerated until used. A plate count of the spore stock was performed 1, 3 and 7 days after preparation to accurately determine the number of spores per ml.

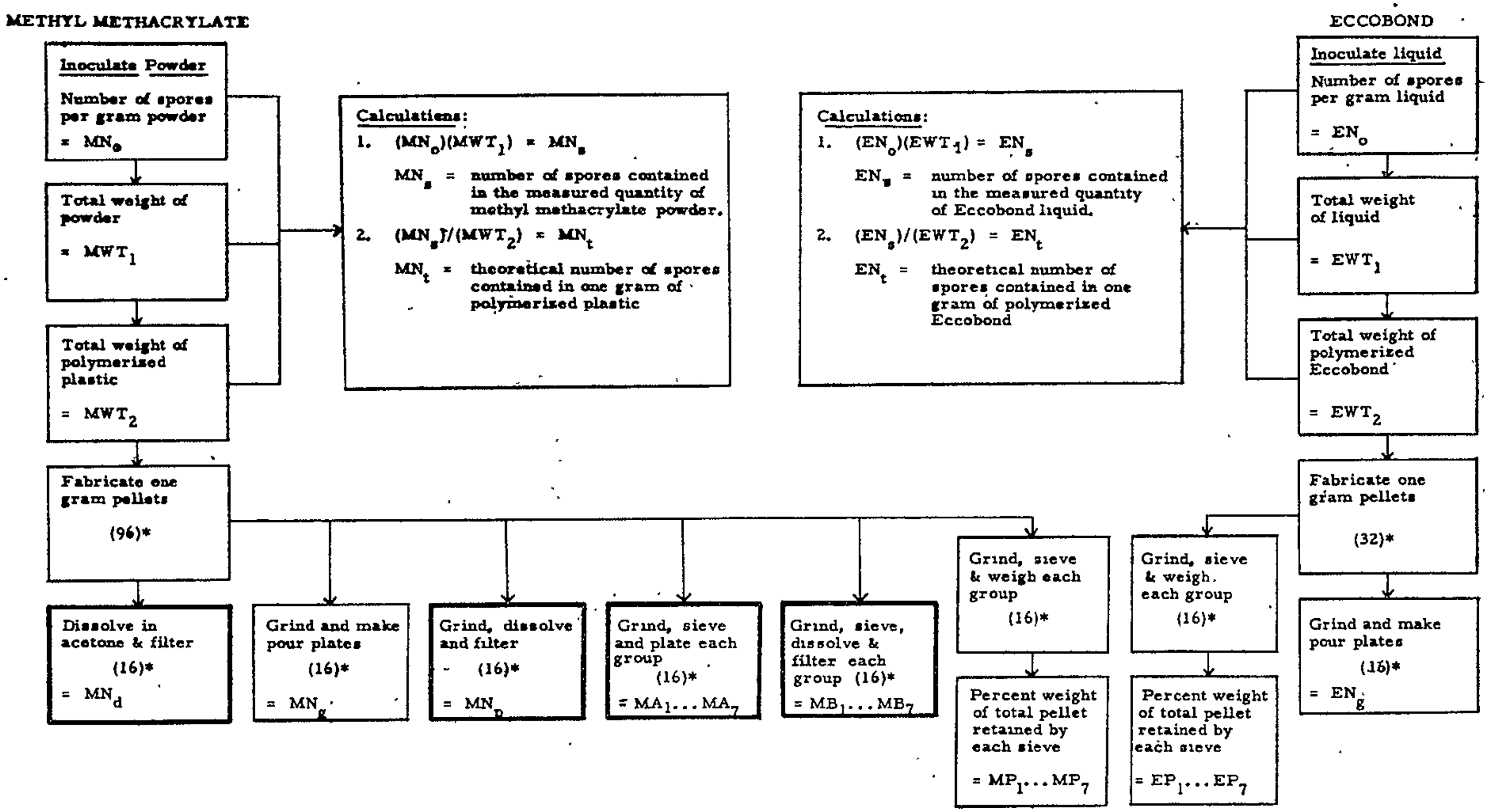
3.1.2 Inoculation of Methyl Methacrylate Powder

Four hundred grams of methyl methacrylate powder were placed in a sterile beaker. The desired number of spores plus enough ethyl alcohol to bring the volume to a total of 400 ml was thoroughly mixed with the powder and air dried in a Class 100 clean bench. The dried seeded powder was sifted through a 40 mesh screen to remove the lumps. All seeded powder was used within one week of preparation.

Prior to using the powder, 0.1 gm aliquots were placed in 100 ml of acetone. After the powder was dissolved, 1 ml aliquots of the solution were filtered through membranes and the membranes placed on TSA. The spore counts obtained in this manner were used to establish the number of spores per gram of methyl methacrylate powder.

3.1.3 Removal of Preservative From Liquid Methyl Methacrylate

One hundred ml of liquid methyl methacrylate were placed in a clean 250



* Number of Replicate Pellets

NOTE: Heavily outlined boxes are additional methyl methacrylate steps

Figure 1: GRINDING EFFICIENCY STUDY SEQUENCE

ml separatory funnel. A 100 ml freshly prepared 2% solution of sodium hydroxide was then added. The mixture was gently swirled for one minute and allowed to separate into two fractions. The bottom pink fraction containing the preservative was discarded. Another 100 ml of 2% sodium hydroxide was added, and the washing process repeated until no pink color was observed in the bottom layer. When the bottom layer was clear, one additional washing with 100 ml sodium hydroxide was performed. The washed liquid methyl methacrylate was rinsed with separate 100 ml volumes of distilled water to remove all traces of sodium hydroxide. The number of rinses was one more than the sodium hydroxide. All water (bottom layer) was drained from the funnel. The washed liquid methyl methacrylate was used within 24 hours of preparation.

3.1.4 Polymerization of Methyl Methacrylate

Forty ml of washed liquid methyl methacrylate were added to 40 gm of seeded powder in a glass beaker and mixed until a uniform slurry was obtained. The mixture was poured into glass test tubes (13 x 100 mm) so that they were 2/3 full. As each tube was filled, the beaker was swirled to assure a homogeneous mixture. When all tubes were filled, they were immediately placed in a desiccator jar and the pressure reduced to 127 mm of mercury for 10 minutes. This vacuum is sufficient to create a slow bubbling action of the mixture in the tubes, but not so low as to cause the mixture to "climb" out the tube as the air is removed by the vacuum. The tubes were then transferred for curing to a 50°C water bath and heated for 1.5 hours. The water level of the bath was adjusted so that it was slightly above the plastic level in the tubes. After 1.5 hours the tubes were removed from the bath and allowed to cool at room temperature for 10 minutes. The fabricated plastic rods were removed by breaking the glass tube. The rods were then stored in a glass jar in the freezer (-18°C) until they were machined into pellets.

3.1.5 Inoculation and Polymerization of Eccobond

One hundred and twenty grams of Eccobond 55 liquid were placed in a glass beaker and the required number of spores in 0.6 ml of ethyl alcohol was added and thoroughly mixed with the Eccobond. Prior to fabricating the solid, 0.1 gm aliquots were removed and placed in 100 ml of acetone. After the liquid Eccobond had dissolved, 1 ml aliquots of the solution were filtered

through membranes and the membranes placed on TSA. The spore counts obtained in this manner were used to establish the number of spores contained in 1 gram of Eccobond 55.

Forty grams of the inoculated Eccobond 55 were added to 4.32 grams of Catalyst 9 and mixed thoroughly. The mixture was then placed in a desiccator jar and the pressure reduced to 127 mm of mercury for 10 minutes to remove the air bubbles. The mixture was then removed from the jar and poured into 13 x 100 mm Teflon tubes which were placed in a 50°C oven for curing. After 3 hours, the tubes were removed from the oven and cooled at room temperature for 10 minutes. The Eccobond was removed from the tubes and the rods were stored in a freezer (-18°C).

3.1.6 Pellet Fabrication

Seeded methyl methacrylate and Eccobond rods were machined into pellets by turning the rods on a lathe. The maching sequence was to cut all rods from one batch of plastic to an established diameter. Face cuts were then made to finish the pellets to the correct length. Each finished pellet was 0.81 cm in diameter, 1.63 cm long, and weighed approximately 1 gm.

3.1.7 Pellet Surface Sterilization

After machining, each pellet was labelled with a number, weighed to the nearest one-thousandth of a gram and this information recorded. Each one was surface sterilized in a freshly prepared 2000 ppm chlorine solution for 10 minutes. This was followed by a 10 minute soak in a fresh filter-sterilized 2% solution of sodium thiosulfate. Then, the pellets were exposed to the airflow of a Class 100 clean bench for 15 minutes to evaporate any moisture on the surface. Each pellet was stored in a freezer in a sterile, appropriately labelled, screw-capped test tube until used.

3.2 PELLET DISSOLVING TECHNIQUE

Acetone was used as the solvent for dissolving methyl methacrylate. Approximately 400 mls of acetone were used to dissolve 1 gram of methyl methacrylate. The acetone and methyl methacrylate were contained in a one liter, screw-capped bottled and placed on a refrigerated reciprocal shaker maintained at 10°C. Approximately 24 hours were required for complete dissolution.

3.3 PELLET GRINDING TECHNIQUE

A Waring blender was modified for pellet grinding by replacing the cutting blade with a flat metal surface to firmly hold an aluminum oxide paper disc (Rub Wet, 240 grit, Armour Star Co.). The cover of the blender jar was modified by drilling a hole through it and inserting a tube down through the blender jar so as to just clear the top of the abrasive disc. A second tube, fitted with a Teflon plug, was used as a push rod to hold a pellet firmly against the abrasive paper. A diagram of the blender is shown in Figure 2.

Following assembly of the blender, 150 ml of sterile distilled water was added to it, the blender placed on the motor and then activated for 1 minute. A 5 ml aliquot of water was then removed and plated in TSA to serve as a sterility control.

After removing the push rod, a pellet was dropped into the tube and the push rod reinserted. The blender was placed on the motor, the motor activated and a slight pressure exerted on the push rod to hold the pellet against the grinding disc. Approximately 10 seconds was required to grind a pellet.

3.4 PARTICLE SIEVING TECHNIQUE

Four wire sieves and three membrane filters were used to size the particles resulting from the grinding process into 7 fractions. United States Standard Testing sieves with sieve number designations of 100, 140, 230 and 400 were used. The size of the sieve openings are 149 μ, 105 μ, 62 μ and 37 μ respectively. Nuclepore filters (General Electric Co.) were used for the membrane filters with pore sizes of 8 μ, 2 μ and 0.5 μ. A schematic flow of the sizing process is shown in Figure 3.

After grinding a pellet, the resulting suspension was poured through the series of sieves which were stacked on top of a one gallon stainless steel pail. The blender was rinsed 2 times with 150 ml aliquots of sterile water and this rinse water was also poured through the sieves. An additional 200 ml of water was then poured through the sieves to ensure removing the particles smaller than 149 μ from the top sieve (No. 100). The particles retained by the 149 μ sieve were rinsed out and collected in a one gallon container. This water and the recovered particles were then filtered through a 0.5 μ membrane

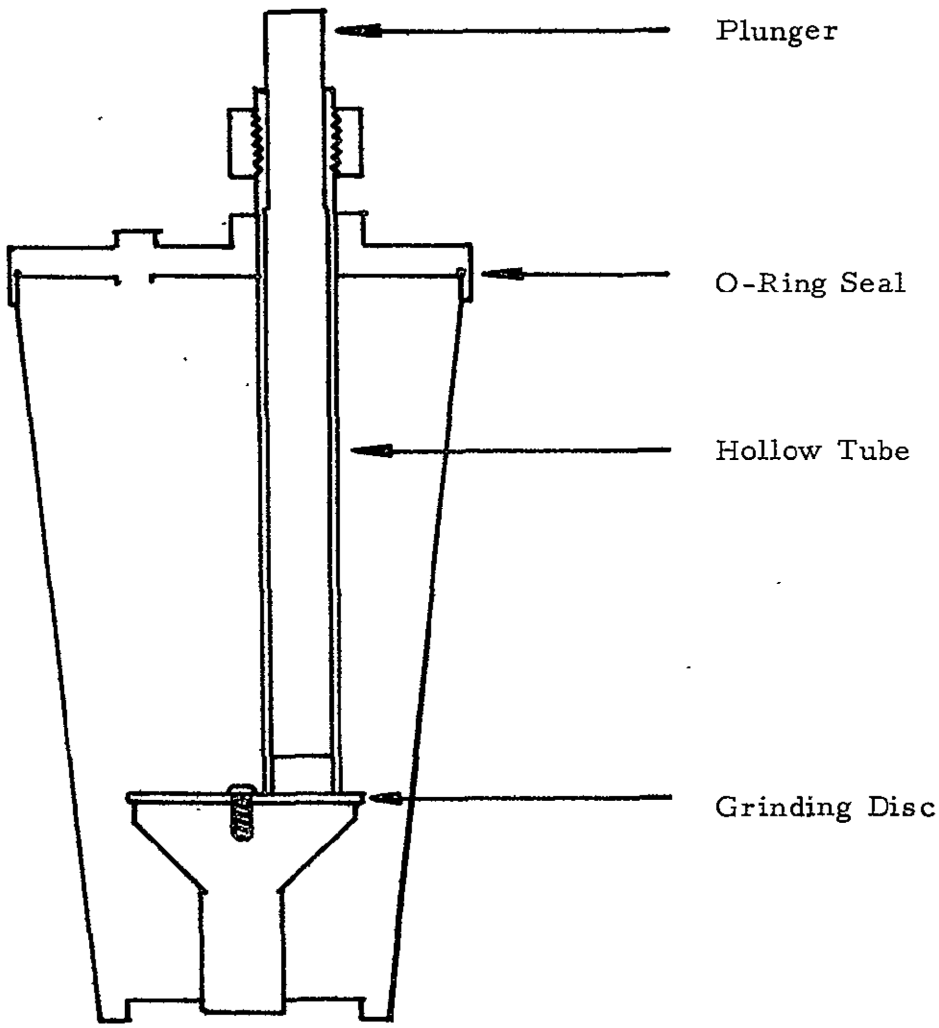


Figure 2 : MODIFIED WARING BLENDER

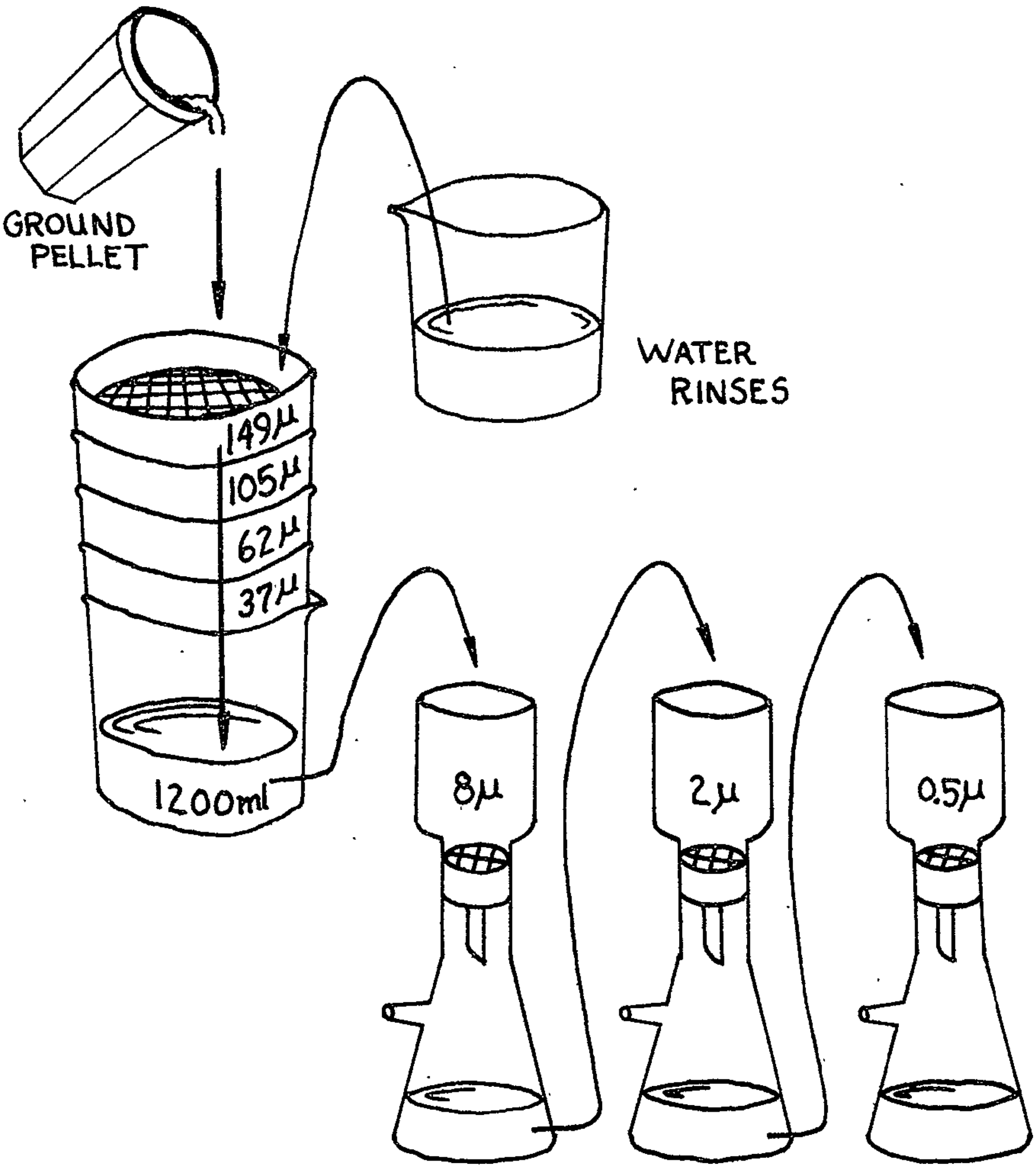


Figure 3: SCHEMATIC OF SIZING PROCESS

filter. The next sieve, 105 μ, in the stack was then rinsed with 200 ml of water and the same process repeated to collect the particles on a 0.5 μ membrane filter. This process was repeated twice more for the remaining two sieves.

Approximately 1200 ml of water containing particles less than 37 μ were collected in the pail from the sieve washings. This suspension was divided into 4 aliquots and each aliquot was filtered through a separate 8 μ filter. The resulting filtrate was then put through a 2 μ filter and finally through a 0.5 μ filter to complete the particle sizing into 7 fractions.

3.5 SPORE RECOVERY ANALYSES

The plating medium used for the spore analyses described in the following paragraphs was Trypticase Soy Agar, BBL. All of the filters used were Nuclepore membrane filters (General Electric Company). The pour plates and filters prepared for enumeration of viable spores were incubated at 30°C for 72 hours prior to colony counting.

3.5.1 Dissolved Methyl Methacrylate

After complete dissolution of a methyl methacrylate pellet in 400 mls of acetone, the solution was assayed for viable spores. A 1 ml aliquot was filtered through a 0.5 μ filter and an additional 10 ml of sterile acetone were used to rinse the filter and filter funnel. Three replicate samples were filtered in this manner and the membranes incubated on TSA. The counts obtained were used to establish the number of spores recoverable from methyl methacrylate pellets by the dissolving technique.

3.5.2 Ground Methyl Methacrylate

Pour plates were made of the suspension resulting from grinding a pellet in 145 ml of water. A 10 ml aliquot was removed from the blender and distributed into 3 separate petri dishes. A large tipped - 10 ml pipette was used for taking the sample because the ground methyl methacrylate tended to clump and plug the opening of regular pipettes. Three replicate samples were plated in this manner for spore counts. These counts represent the number of spores recoverable by grinding methyl methyacrylate pellets.

3.5.3 Ground and Dissolved Methyl Methacrylate

After grinding a pellet, the suspension was filtered through a 0.5 μ filter. The blender was rinsed twice with 150 ml aliquots of water and the rinse water was also filtered. The filter and particles trapped on it were put into a Fernbach culture flask and 400 ml of acetone added. After complete dissolution of the particles, a 2 ml aliquot was removed and filtered through a 0.5 μ filter. The filter was then rinsed with 10 ml sterile acetone and plated on TSA. Three replicate samples were prepared and the filters incubated for viable spore counts. The counts obtained in this manner represent the number of spores that have survived the abrasiveness of grinding.

3.5.4 Ground and Sieved Methyl Methacrylate

Methyl methacrylate pellets were ground and sieved into 7 fractions as described in Section 3.4. The four 0.5 μ filters used to collect the particles from the 100, 140, 230 and 400 wire screens were each placed in a separate small screw-capped bottle containing 20 ml of sterile distilled water. The four filters used to collect the 8 u particles were also put into a bottle containing 20 ml of water. Pour plates were made from aliquots of each of the samples to obtain the spore counts associated with each fraction. The filters containing the 2 μ and 0.5 μ particles were plated directly in TSA. These last two fractions contained very few spores, therefore, dilution in water was unnecessary.

3.5.5 Ground, Sieved and Dissolved Methyl Methacrylate

The filters used to collect the particle fractions after grinding and sieving a pellet were placed in individual 250 ml Erlenmeyer flasks, each containing 100 ml sterile acetone. The four filters containing the 8 μ particles were placed in one flask. After complete dissolution of the particles, aliquots from each sample were filtered through 0.5 μ filters. Each filter was rinsed with 10 ml acetone and then plated in TSA. The counts obtained were used to establish the total number of spores present in each fraction.

3.5.6 Ground Eccobond

Viable spore counts from ground Eccobond pellets were obtained in the same manner as for methyl methacrylate described in Paragraph 3.5.2.

3.5.7 Ground and Sieved Eccobond

Viable spore counts from the sieved fractions of ground Eccobond are the same as described in Paragraph 3.5.4.

3.6 PARTICLE DISTRIBUTION

The distribution of particle sizes resulting from grinding methyl methacrylate and Eccobond was determined by weight of the particles in each fraction after sieving, see Section 3.4. The filters used to collect the particles from the sieving process were weighed prior to use. After collecting the particle fractions on a filter, the filter was placed in a petri dish and dried in an oven at 50°C for 72 hours. After drying the particles, the filter was again weighed to obtain the weight of the material collected.

4.0 RESULTS AND DISCUSSION

4.1 SPORE RECOVERY FROM PARALLEL STUDIES

A summary of the results obtained from the parallel studies conducted with methyl methacrylate and Eccobon is presented in Table 1. The unreduced data from which these summary figures were calculated are given in the Appendix.

Table 1: SUMMARY OF PARALLEL STUDIES

Material	Average Theoretical No. Spores per Gram	Average No. Spores per Gram by Dissolving	Average No. Spores per Gram by Grinding	Average No. Spores per Gram by Grinding/Dissolving
Methyl Methacrylate	50,200	33,600	1500	6500
Eccobond	39,800	Data not Obtainable	1600	Data not Obtainable

The theoretical number of spores for methyl methacrylate was calculated from the spore counts obtained from the inoculated powder. These counts showed an average of 7.0×10^4 spores per gram of powder. Forty grams of powder were used to make each batch of plastic which represented 2.8×10^6 spores per batch. One batch of polymerized plastic weighed 56 grams which results in 5.0×10^4 spores per gram of methyl methacrylate.

The theoretical spore level in Eccobond was derived in approximately the same manner. The spore counts obtained from the inoculated Eccobond 55 showed an average of 4.5×10^4 per gram of material. Thirty-six grams of Eccobond 55 were used to make each batch of epoxy giving a total of 1.6×10^6 spores per batch. The total weight of a polymerized Eccobond batch was 40.32 grams which results in 4.0×10^4 spores per gram of epoxy. These derived theoretical spore numbers for methyl methacrylate and Eccobond represent the number of viable spores that should be present in a one-gram pellet. This assumes that no die-off occurred during the polymerization process or during the fabrication of the pellets.

By comparing the data given in Table 1, the following calculations are made. A visual presentation of the relationships is presented in Figure 4.

4.1.1 Methyl Methacrylate

1) The number of spores killed during polymerization and pellet fabrication are calculated by comparing the theoretical number of spores with the spore counts obtained from dissolving one gram pellets. This amounts to a reduction factor of 1.5. (A reduction factor is the ratio of one spore count to another spore count, for example $50,200 \div 33,600 = 1.5$).

2) The number of spores killed by the abrasiveness of grinding is derived by comparing the dissolved spore counts with the spore counts obtained by grinding the pellets and then dissolving the resulting particles. This calculation gives a reduction factor of 5.2.

3) The number of viable spores that are uncountable after grinding because they are still encapsulated in particles is calculated by

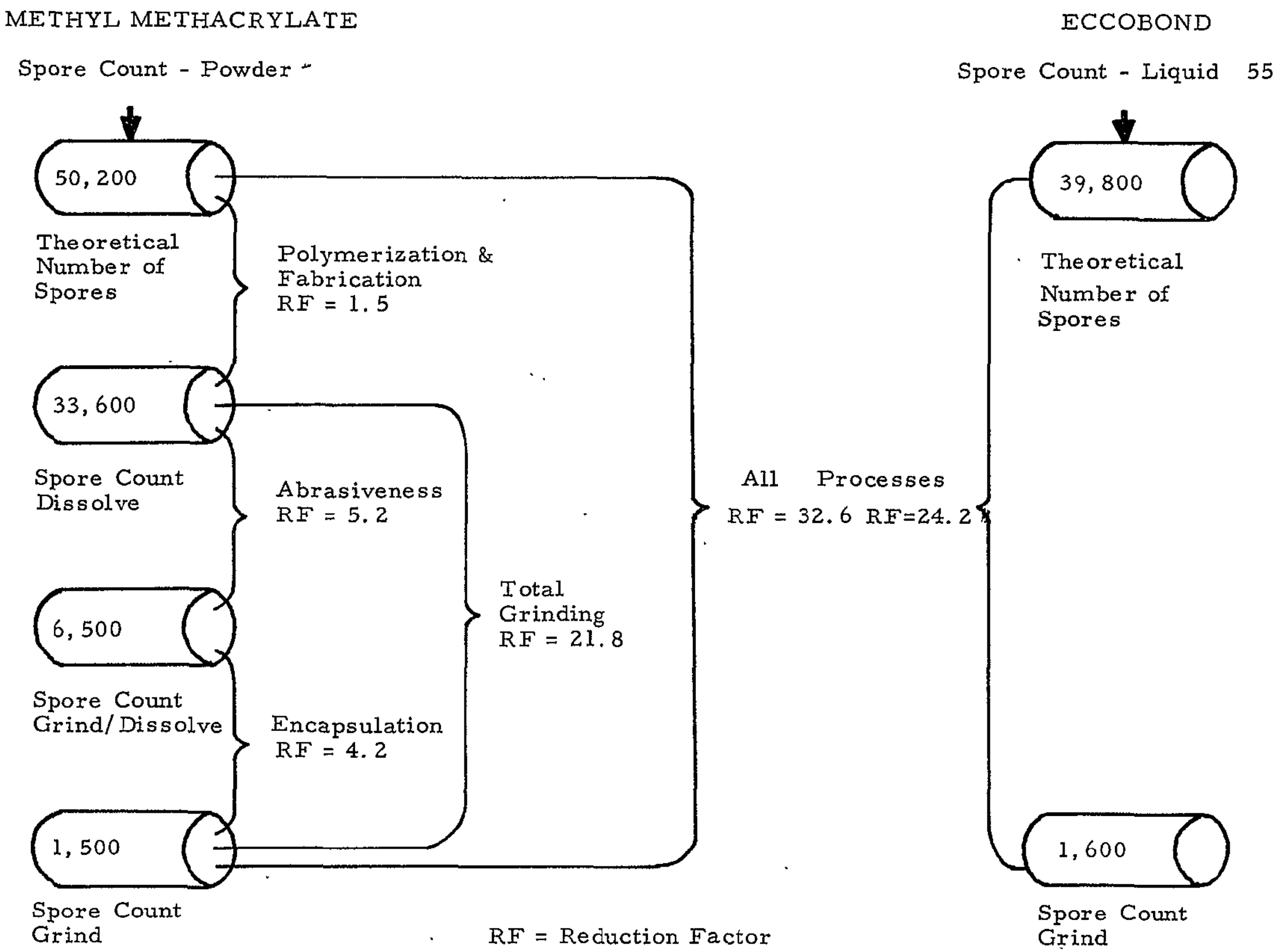


Figure 4: SUMMARY OF REDUCTION FACTORS

comparing the grinding/dissolving counts to the grinding counts. This amounts to a reduction factor of 4.2.

4) The number of spores that are lost due to the total grinding process is obtained from the ratio of dissolved counts to the grinding counts. This calculation shows a reduction factor of 21.8.

5) A total reduction in spore numbers due to all processes, i.e., polymerization, pellet fabrication, abrasiveness of grinding and viable spores which remain encapsulated in particles, is found by comparing the theoretical number of spores to the spore counts obtained by grinding. This total reduction factor is 32.6.

4.1.2 Eccobond

Since there is not a suitable solvent for Eccobond, the only method readily available for obtaining spore counts is grinding. Therefore, only the total spore reduction factor can be calculated by comparing the theoretical number of spores with the counts obtained by grinding. This reduction factor is 24.2.

4.2 GRINDING ADJUSTMENT CONSTANT

The most accurate method currently available for recovering viable spores from solids is by dissolution. Significant reductions are observed in the number of viable spores when the grinding technique is used. Therefore, it becomes necessary to adjust the spore counts obtained by grinding. A grinding adjustment constant can be derived from the reduction factors.

An appropriate analysis of variance method was used to compare the total reduction factors for methyl methacrylate (32.6) and Eccobond (24.2). The results show no significant difference ($F = 2.04$ with 1 and 30 degrees of freedom) between the total reduction factors. On the one hand then, assuming the same die-off for spores in Eccobond due to polymerization and pellet fabrication as there is in methyl methacrylate, the adjustment constant would be 16. However, if no die-off occurs during polymerization and pellet fabrication in Eccobond the adjustment constant would be 24.2. It appears that an average of these two figures more closely reflects the true picture. Therefore, 20 is selected for use as the grinding adjustment constant.

4.3 PARTICLE SIZES FROM GROUND PELLETS

Pellets of methyl methacrylate and Eccobond were ground and sieved into seven fractions. The particles in each fraction were dried and weighed to determine the amount of pellet retained for each pore size. A summary of the percent weight of the total pellet recovered for each fraction is given in Table 2. The raw data for these summary figures are presented in the Appendix.

Table 2: PERCENT WEIGHT OF PARTICLES RECOVERED IN EACH FRACTION AFTER GRINDING PELLETS

Material	Wire Sieve Pore Size				Filter Pore Size		
	149 µ	105 µ	62 µ	37 µ	8 µ	2 µ	0.5 µ
Methyl Methacrylate	67	4	5	6	19	0	0
Eccobond	22	6	5	15	51	<0.1	<0.1

A comparison of the grinding characteristics for the two materials show that over 60 percent of the methyl methacrylate particles are larger than 149 µ and approximately 50 percent of the Eccobond particles are between 37 µ and 8 µ. A cursory microscopic examination of the particles indicate that many of the methyl methacrylate particles are long thin strands while those of Eccobond appear to be much shorter with approximately the same diameter. It is possible that some of the shorter Eccobond strands washed through the larger sized sieves which could account for the wide variance in percent retention for the two materials. Sieved particles of the two materials have been sent to JPL for a more detailed examination by electron microscopy.

4.4 PERCENT SPORE OUTGROWTH

One gram pellets of methyl methacrylate were ground and then sieved into 7 fractions. Plate counts were made from each fraction to determine the number of spores that were exposed sufficiently to allow outgrowth.

A second set of pellets were ground and sieved into 7 fractions. The particles collected in each fraction were then dissolved in acetone and counts made to determine the total number of spores that were present in each fraction. From these data, the percent spore outgrowth for each particle fraction was calculated, see Table 3. The data shows that as the particle size decreases the percent of spore outgrowth increases with 100 percent outgrowth occurring with particle sizes between 8 μ and 2 μ.

One gram pellets of Eccobond were also ground and sieved into 7 fractions. Plate counts were made from the particles for each fraction to obtain the number of exposed spores. The total number of spores actually present in the particles for each fraction can be computed by inference from the methyl methacrylate data. If the assumption is made that spore outgrowth is a direct function of particle size, the total number of spores in each fraction can be calculated from the percent outgrowth figures obtained for methyl methacrylate as given in Table 3. However, another assumption can be made, that is, the total number of spores contained in the particles of each fraction is a function of the percent weight of the particles retained in each fraction. A summary of these computed total number of spores in Eccobond is given in Table 4.

Table 3: SPORE OUTGROWTH AS A FUNCTION OF PARTICLE SIZE IN GROUND METHYL METHACRYLATE

Pore Size μ	Mean Number of Spores Recovered from Grinding and Sieving	Mean Number of Spores Recovered from Grinding, Sieving and Dissolving Particles	% Spore Outgrowth from Sieved Particles
149	299	4193	7.1
105	32	209	15.3
62	25	137	18.2
37	58	235	24.7
8	913	1214	75.2
2	15	9	100+
0.5	31	18	100+
Total Number of Spores	1373	6015	

Table 4: ESTIMATED TOTAL NUMBER OF SPORES IN GROUND ECCOBOND

Pore Size μ	Mean Number of Spores Recovered From Grinding and Sieving Eccobond	Computed Total Number of Spores Based on % Spore Outgrowth for Methyl Methacrylate	Computed Total Number of Spores Based on % Weight for Each Size for Eccobond
149	238	3352	1382
105	135	882	377
62	81	445	314
37	182	737	942
8	794	1056	3703
2	3	3	3
0.5	1	1	1
Total Number of Spores	1434	6476	6222

5.0 SUMMARY AND RECOMMENDATIONS

A series of parallel studies were conducted to determine the efficiency of a grinding technique to recover B. subtilis var. niger spores from internally inoculated methyl methacrylate and Eccobond. Pellets of the two materials were inoculated and fabricated to contain approximately 5×10^4 spores per gram. Dissolving and grinding techniques were employed to recover the spores from methyl methacrylate and reduction factors for spore recovery were determined. The enumeration of spores in Eccobond was obtained by grinding only. From these data, the reduction in spore recovery from grinding Eccobond was determined. An adjustment constant of 20 was calculated for the grinding process.

The parallel studies showed that there are approximately 20 times more viable spores present in a one-gram pellet of Eccobond than the counts indicate from grinding. Therefore, it is recommended that the initial spore levels obtained for Eccobond in the previous investigation (JPL Contract No. 952511, "Release of Microorganisms From Solids After Simulated Hard Impact," Test III) be increased by a factor of 20. This increase in initial spore levels for Eccobond will result in decreasing the calculated percentage of spores released after impact onto stainless steel. These adjustments will make the Eccobond data more consistent with the methyl methacrylate data on the percentage of microbial release from solids.

It is also recommended that the initial spore levels obtained for Eccobond in Phases II and III of this investigation (JPL Contract No. 952916, "Release of Microorganisms From Solid Materials") be increased by a factor of 20.

6.0 APPENDIX

The unreduced data for Phase I are presented in Tables 5 through 10.

Table 5: NUMBER OF SPORES RECOVERED FROM ONE GRAM OF MATERIAL

Replicate Samples	Methyl Methacrylate			Eccobond
	MN_d *	MN_g *	MN_p *	EN_g *
1	35334	907	5784	804
2	29727	1612	4491	1628
3	32880	1859	6055	863
4	33383	1517	6054	1978
5	33940	1818	5138	1791
6	31325	1852	9127	1784
7	29682	2416	6793	1922
8	34278	1928	6162	540
9	29026	1664	6970	2302
10	32704	1831	5464	2465
11	33323	1471	6246	1287
12	35098	1215	8655	1822
13	36487	1094	5882	2224
14	33574	815	6494	1943
15	38453	1520	8114	1366
16	38666	1136	5986	1595
Mean	33618	1541	6463	1645

* MN_d = Spores recovered by dissolving methyl methacrylate.

MN_g = Spores recovered by grinding methyl methacrylate.

MN_p = Spores recovered by grinding methyl methacrylate, then dissolving particles.

EN_g = Spores recov ered by grinding Eccobond.

Table 6: PERCENT WEIGHT OF PARTICLES RECOVERED FROM EACH FRACTION AFTER GRINDING AND SIEVING METHYL METHACRYLATE PELLETS

Replicate Samples	Wire Sieve Pore Size				Filter Pore Size		
	149 μ	105 μ	62 μ	37 μ	8 μ	2 μ	0.5 μ
1	60	4	6	8	22	0	0
2	52	5	6	10	27	0	0
3	73	3	4	5	15	0	0
4	70	3	4	6	17	0	0
5	70	4	4	5	17	0	0
6	79	2	3	4	12	0	0
7	70	4	4	6	17	0	0
8	67	3	4	6	21	0	0
9	64	4	6	7	19	0	0
10	69	3	5	6	18	0	0
11	64	4	5	7	21	0	0
12	74	3	4	5	15	0	0
13	70	3	4	5	17	0	0
14	61	5	5	8	21	0	0
15	64	5	5	7	19	0	0
16	61	5	6	8	21	0	0
Mean	67	4	5	6	19	0	0

Table 7: PERCENT WEIGHT OF PARTICLES RECOVERED FROM EACH FRACTION AFTER GRINDING AND SIEVING ECCOBOND PELLETS

Replicate Samples	Wire Sieve Pore Size				Filter Pore Size		
	149 μ	105 μ	62 μ	37 μ	8 μ	2 μ	0.5 μ
1	27	13	5	10	45	0.1	0
2	22	29	7	16	25	0	0
3	25	13	6	16	40	0	0
4	16	7	5	37	36	0	0
5	27	2	6	17	49	0	0
6	31	3	5	11	49	0	0
7	31	2	5	20	42	0	0
8	21	3	5	23	48	0	0
9	22	2	5	13	58	0	0
10	13	2	5	12	68	0	0
11	22	2	5	12	59	0.1	0.1
12	23	3	5	10	60	0.1	0
13	19	3	6	11	62	0	0
14	27	3	5	11	54	0	0
15	18	3	5	12	62	0	0
16	14	3	5	13	65	0	0
Mean	22	6	5	15	51	<0.1	<0.1

Table 8: NUMBER OF SPORES RECOVERED FROM EACH FRACTION AFTER GRINDING AND SIEVING ONE GRAM OF METHYL METHACRYLATE

Replicate Samples	Wire Sieve Pore Size				Filter Pore Size			Total Spores
	149 μ	105 μ	62 μ	37 μ	8 μ	.2 μ	0.5 μ	
1	221	36	15	53	615	9	15	964
2	266	15	17	11	988	24	14	1335
3	308	13	19	71	972	38	33	1454
4	327	56	21	86	984	4	63	1541
5	400	53	47	90	1056	13	58	1717
6	356	45	28	99	732	14	24	1298
7	239	26	28	19	969	11	22	1314
8	291	17	13	68	973	31	35	1428
9	384	40	34	76	998	15	49	1596
10	316	38	25	49	763	14	19	1224
11	211	45	28	47	893	4	29	1257
12	284	19	32	70	1024	2	27	1458
13	291	43	21	68	730	3	21	1177
14	356	40	28	72	1541	24	56	2117
15	329	21	32	47	730	21	12	1192
16	203	11	13	2	646	9	18	902
Mean	299	32	25	58	913	15	31	1373

Table 9: NUMBER OF SPORES RECOVERED FROM EACH FRACTION AFTER GRINDING, SIEVING AND DISSOLVING THE PARTICLES FROM ONE GRAM OF METHYL METHACRYLATE

Replicate Samples	Wire Sieve Pore Size				Filter Pore Size			Total Spores
	149 μ	105 μ	62 μ	37 μ	8 μ	2 μ	0.5 μ	
1	3896	214	90	141	723	1	3	5068
2	3086	173	99	106	282	2	1	3749
3	5403	289	150	182	1658	19	24	7725
4	4054	242	125	203	891	6	32	5553
5	3615	238	168	324	1655	10	16	6026
6	4125	204	222	321	1538	13	20	6443
7	2439	180	113	127	673	5	10	3547
8	3700	188	109	127	584	4	17	4729
9	3845	216	124	152	819	10	15	5181
10	4300	234	156	322	1370	14	18	6414
11	3275	241	159	199	1433	6	10	5323
12	4763	184	124	347	1396	4	27	6845
13	5430	223	117	234	2033	20	32	8089
14	4891	268	180	516	2385	15	43	8298
15	5384	74	120	213	941	5	12	6749
16	4879	182	139	243	1035	11	14	6503
Mean	4193	209	137	235	1214	9	18	6015

Table 10: NUMBER OF SPORES RECOVERED FROM EACH FRACTION AFTER GRINDING AND SIEVING ONE GRAM OF ECCOBOND

Replicate Samples	Wire Sieve Pore Size				Filter Pore Size			Total Spores
	149 µ	105 µ	62 µ	37 µ	8 µ	2 µ	0.5 µ	
1	364	114	50	91	1140	13	1	1773
2	502	205	50	66	1028	6	4	1861
3	158	263	147	214	610	4	2	1398
4	187	73	56	17	541	1	0	875
5	135	97	104	158	739	0	1	1234
6	205	45	50	67	396	2	1	766
7	215	104	83	351	789	1	1	1544
8	256	153	87	193	633	2	0	1324
9	63	237	19	177	629	0	1	1126
10	344	206	42	127	657	3	0	1379
11	184	59	104	354	1484	1	1	2187
12	198	123	146	223	1021	1	0	1712
13	362	160	48	156	754	2	1	1483
14	198	132	81	200	790	3	0	1404
15	237	133	119	217	661	3	1	1371
16	201	59	116	293	839	2	1	1511
Mean	238	135	81	182	794	3	1	1434

SECTION III

SECTION III

PHASE II

IMPACTION OF INOCULATED ECCOBOND ONTO SAND

TABLE OF CONTENTS

PHASE II

IMPACTION OF INOCULATED ECCOBOND ONTO SAND

1.0 PURPOSE

This investigation was conducted to determine the percentage release of microorganisms embedded in pellets that were impacted onto sand.

2.0 INTRODUCTION

Eccobond pellets were fabricated so that each pellet interior contained approximately 10^4 Bacillus subtilis var. niger spores. A series of these pellets were propelled from a gun at 168, 457, 945 and 1554 meters per second into sand. After sand impact, the pellet fragments were analyzed to determine the number of exposed viable microorganisms. The test program is presented in Table 1.

3.0 PROCEDURES

3.1 PELLET MANUFACTURE

3.1.1 Preparation of Spore Stock

A 0.1 ml aqueous suspension containing 10^8 B. subtilis spores was placed in the bottom of sterile planchets. The stock spore suspension was a minimum of 6 months old. The planchets were dried overnight by airflow from a Class 100 clean bench. When all the water had evaporated, 10 planchets were placed in a bottle containing 30 ml ethanol and insonated for 20 minutes. The level of the ultrasonic bath water was adjusted so that it was half way up the side of the bottle. After insonation, the 30 ml spore-ethyl alcohol suspension was pipetted into a sterile, capped bottle. All planchets were rinsed in fresh ethyl alcohol and the washings plus additional alcohol were combined with the 30 ml stock to total 100 ml. The stock inoculum was refrigerated until used. A plate count of the spore stock was performed 1, 3, and 7 days after preparation to accurately determine the number of spores per ml.

3.1.2 Epoxy Fabrication

Thirty-six grams of Eccobond 55 were placed in a glass beaker and 4.32

Table 1: PHASE II - ECCOBOND PELLETS IMPACTED ONTO SAND

Pellet Launching Velocity, Meters/Second	Percent, by Weight, Pellet Recovered After Impact (sterile pellets)	Total Spores Released from Impacted Pellet (10^4 spores/pellet)	Procedural Controls (sterile pellets)
168	6 [(a)]	6	1
457	6	6	1
945	6	6	1
1554	6	6	1
	24	24	4

(a) Number of Replicate Pellets

<u>52 Total Pellets Fired</u>

grams of Catalyst 9 added. A 6×10^6 spore inoculum in 0.2 ml of alcohol was added and the mixture thoroughly stirred. The glass beaker containing the liquid Eccobond was placed in a desiccator jar and the pressure reduced to 127 mm of mercury for 10 minutes. The mixture was removed from the jar and poured into 13 x 100 mm teflon tubes. The tubes were cured in a 50°C oven for 3 hours.

After curing, the tubes were removed from the oven, allowed to cool at room temperature for 10 minutes, and the Eccobond removed. The Eccobond rods were stored in a glass jar, labelled with information as to date of seeding, fabrication, batch number, and the number of spores per gram of epoxy.

3.1.3 Pellet Machining

A seeded Eccobond rod was machined into firing pellets by turning the rod on a small bench lathe. Each finished pellet was approximately 0.81 cm in diameter, 1.63 cm long, and weighed approximately 1.0 gram. The machining sequence was to cut all rods of the same epoxy batch to the established diameter. Face cuts were then made to finish the pellets to the correct length.

3.1.4 Pellet Surface Sterilization

After machining, each pellet was identified by a number, weighed to the nearest one-thousandth of a gram and this information recorded. Each pellet was surface sterilized in a freshly prepared 2000 ppm chlorine solution for 10 minutes. This was followed by a 10 minute soak in a fresh filter-sterilized 2% solution of sodium thiosulfate. Each pellet was stored in a sterile, appropriately labelled, screw-capped test tube until launched.

3.2 PELLET LAUNCHINGS

3.2.1 Collection Canister

The sterile collection canister (Figure 1) was constructed such that an impacting pellet struck only sand. In order to facilitate recovery of the projectile particles and impacted sand, the impact area was isolated from

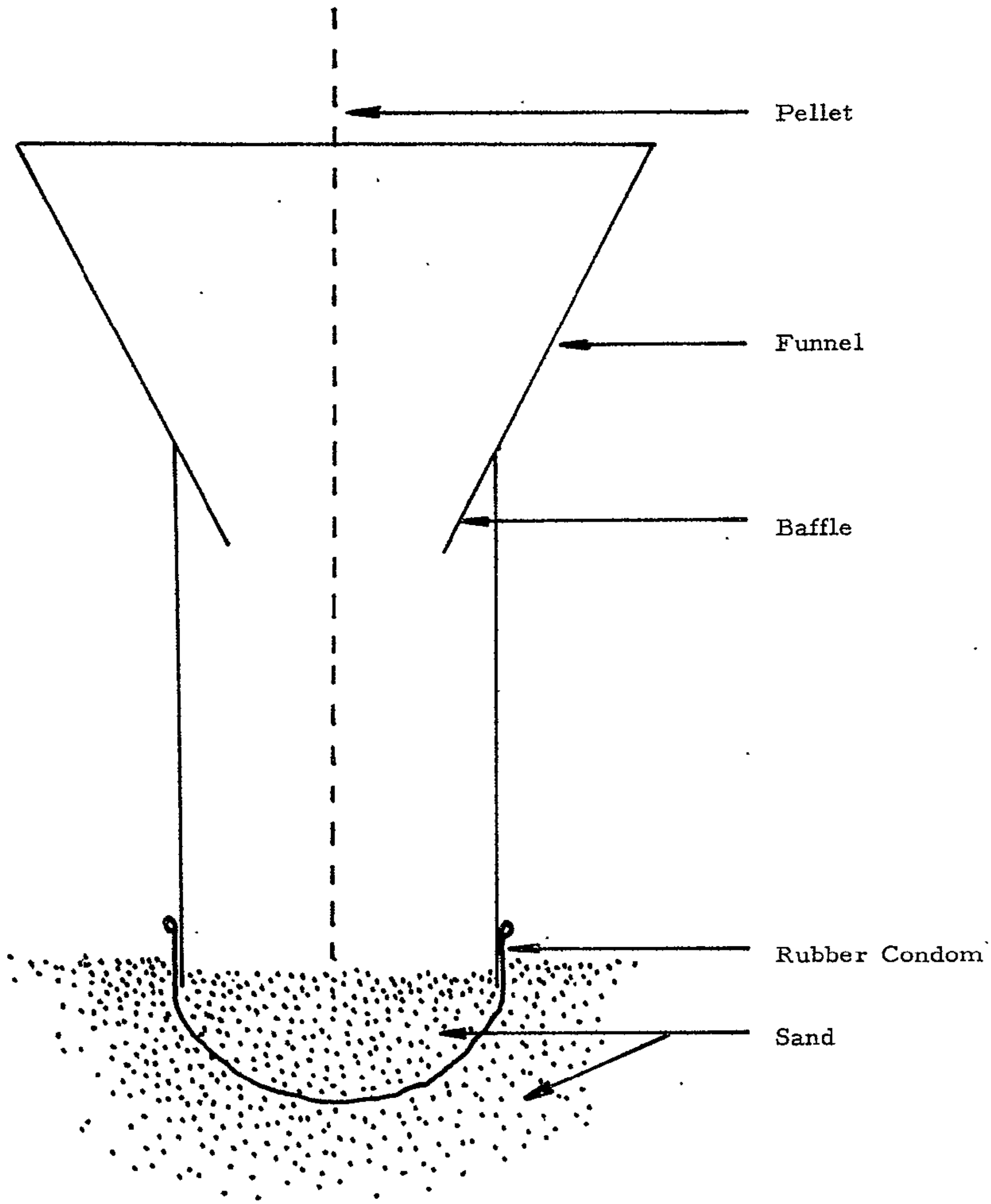


Figure 1: COLLECTION CANISTER FOR SAND IMPACT

the rest of the supporting sand by a rubber condom. With this apparatus, it was possible to work with a relatively small quantity of sand (less than 200 grams) without changing the impact characteristics of the sand. A baffle was also incorporated in the collection device to aid in retaining the sand at impact.

3.2.2 Pellet Firings

The pellets were fired by personnel of the Damage Mechanics Laboratory. In order to assure reliability in the firings, one person was responsible for all launchings. The gun barrel was positioned so that impact occurred vertically into the sand in an upright canister (Figure 2). The gun was cleaned before each day's firing by first flushing the barrel and chamber with air followed by an ethanol scrub. The sterile collection canister containing the sand was placed in the gun chamber in the support sand. The pellet was loaded in the breech using sterile forceps. The cover of the canister was removed, the chamber door closed, and the pellet launched. After firing, the chamber door was opened, the canister cover replaced, the velocity recorded, and the canister returned to the Microbiology Laboratory for analysis.

The Eccobond pellets were fired at velocities of 168 ($\pm$ 30), 457 ($\pm$ 60), 945 ($\pm$ 90), and 1554 ($\pm$ 90) m/sec. Compressed air was employed to attain 168 m/sec, and a powder charge was used to launch pellets at the higher velocities. Velocities were measured using breakscreen techniques.

3.3 PELLET ANALYSIS

3.3.1 Percent Pellet Recovery After Impact

Six unseeded Eccobond pellets were launched at each test velocity and impacted on sand. Each pellet was weighed, fired and collected in a separate canister. All particles of the impacted pellet were then weighed again. The procedures used to recover the particles were identical to those that were employed in the analysis for released spores from seeded pellets after impact. The results of these firings established the percent of pellet recovered. The particles of each impacted pellet were saved for delivery to JPL.

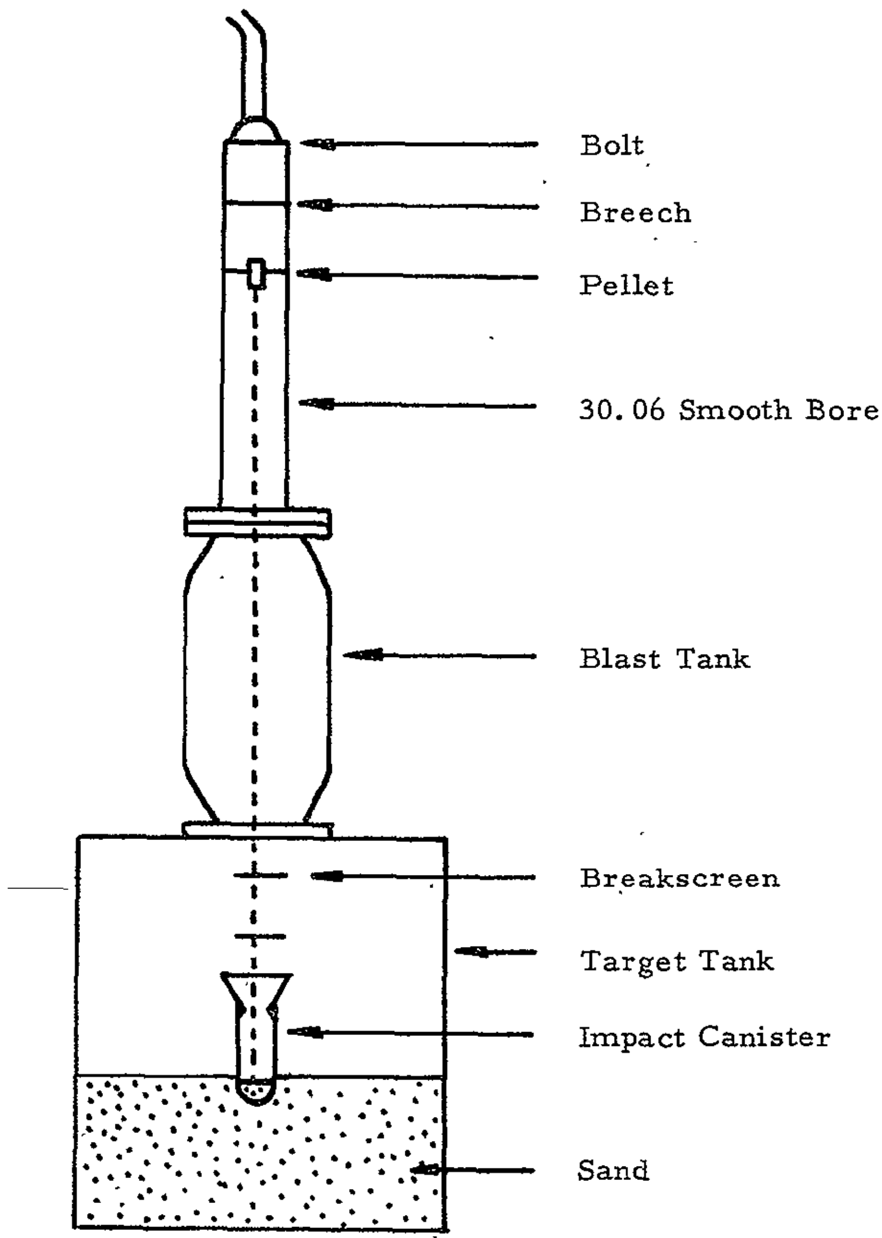


Figure 2: GAS AND POWDER GUN

3.3.2 Analysis for Spores Released After Pellet Impact

The collection canister, containing the impacted pellet, was analyzed in the Microbiology Laboratory. Analysis for the recovery of released spores was performed as outlined in Figure 3. The rubber condom containing the plastic particles and the impacted sand was removed from the canister. Large projectile particles were recovered by sifting the sand through a sterile screen mounted over a sterile beaker. The trapped pellet particles were transferred, with sterile forceps, to a sterile petri plate. Trypticase Soy Agar (TSA) was then poured into the plate so that each particle was completely covered with the medium. The sifted sand was poured into plates of molten TSA and gently swirled to insure even particle distribution. The sand and particles were incubated in TSA for 2 weeks at 30°C. Daily examinations were performed and each colony observed was recorded as one released spore.

3.3.3 Analysis for Total Spore Level

On each day of firing two pellets were chosen at random to determine the total spore level. A pellet was placed in a modified Waring blender (Figure 4), and wet ground. The contents of the blender were plated in molten TSA and incubated for 2 weeks at 30°C. The number of spores recovered by this procedure was multiplied by 20 to account for the spore reduction which occurs by grinding as discussed in Section II of this report. This procedure established the number of spores present per gram of pellet.

3.3.4 Procedural Controls

On each test day, an unseeded control pellet was launched prior to the firing of seeded pellets. The control pellet particles were subjected to analysis as outlined in Figure 3. This control pellet established the reliability of the test data with respect to possible contamination due to procedural techniques.

Also, on each test day, a seeded pellet was selected at random and embedded in melted TSA. The plate was incubated at 30°C and examined periodically for 2 weeks. The absence of surface colonies established reliability in the method used to sterilize the pellet surface.

PELLET FABRICATION

Pellet Firing

168, 457, 945 and 1554 Meters/Second

Sand Impact

Analysis for Total Spore Level

1. Wet grind pellet in modified Waring blender.
2. Plate aliquots of ground Eccobond in TSA.
3. Incubation for 2 weeks at 30°C.
4. Plate count at 72 hours and 2 weeks.

Analysis for Spores Released After Impact

1. Rubber condom removed.
2. Particles removed from sand by sifting through a sterile screen.
3. Particles, sand, and membrane plated separately in TSA.
4. Incubation for 2 weeks at 30°C.
5. Daily examination.

Figure 3: TEST ANALYSIS

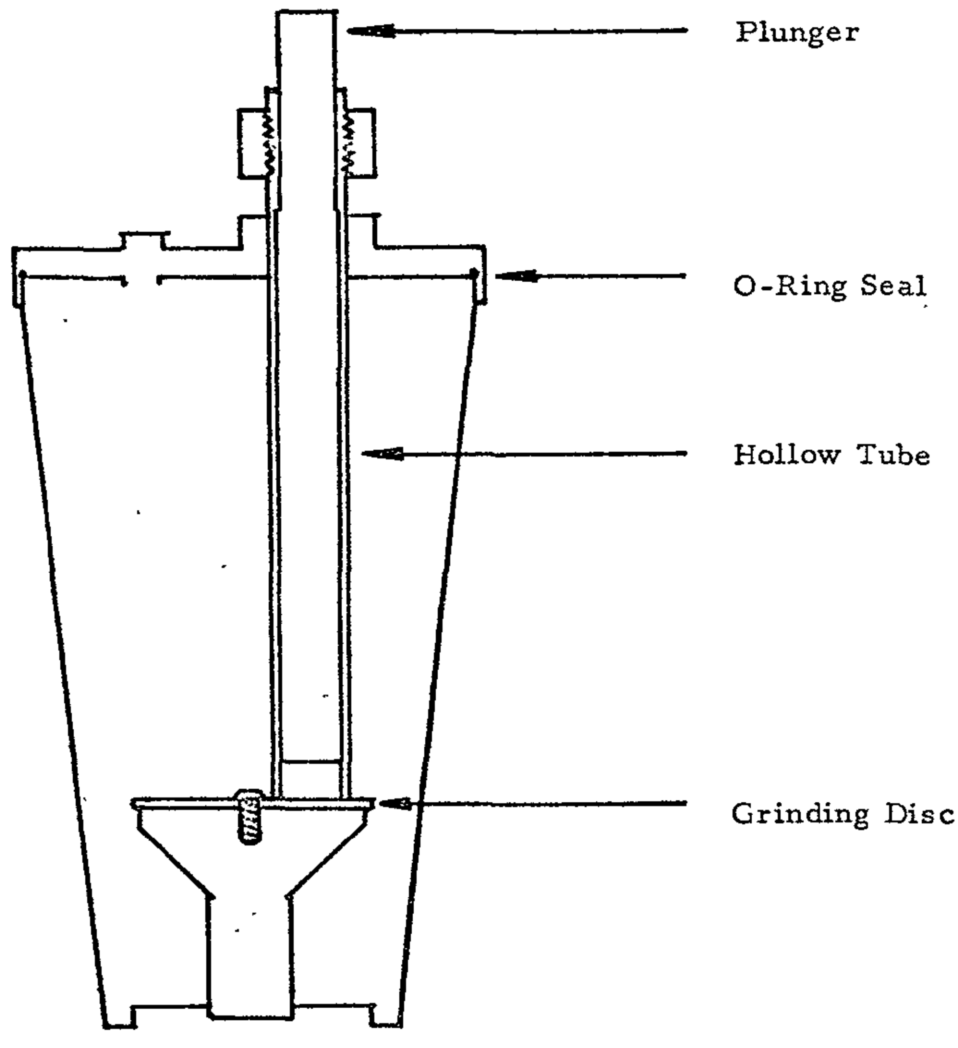


Figure 4 : MODIFIED WARING BLENDER

3.4 DATA RECORDING AND ANALYSIS

The data was recorded on data sheets that contained the date of pellet firing, pellet number and weight, the velocity of impact, the analysis performed and the results of the analysis. The data sheet for each test day was checked and initialed by the program manager.

These data provided information on: (1) the number of viable organisms released from solids after hard impact; (2) differences in release of organisms due to variations in impact velocities.

The data were evaluated statistically using analysis of variance techniques. These analyses detected any significant variance between pellets in a replicate group and between pellets impacted at 168, 457, 945 and 1554 m/sec.

4.0 RESULTS AND DISCUSSION

4.1 PERCENT PELLET RECOVERED AFTER IMPACT AND FRACTURE CHARACTERISTICS

The percentage of epoxy recovered from the sand after pellet impaction at the four test velocities is presented in Table 2. The unreduced data is given in the Appendix.

Table 2: PERCENT RECOVERY OF PELLET FOLLOWING IMPACT

Velocity m/sec	Mean Percent Recovery of Pellet (a)
168	100
457	92
945	73 (b)
1554	69 (b)

(a) Mean of 6 Pellets

(b) Many small "sand-grain" size pellet particles passed through the recovery sieve with the sand.

A majority of the pellets launched at 168 m/sec did not fracture upon impact in the sand. However, areas of sand abrasion were observed on thèse intact pellets. The higher velocities, 457, 945 and 1554 m/sec, resulted in completely fractured pellets. As the impact velocity increased, the number of pellets particles increased and the size of the pieces decreased.

In fact, fractured pellet pieces the size of sand grains accounted for approximately 30%, by weight, of a pellet impacted at 945 and 1554 m/sec. Figures 5 through 8 show a comparison of a pellet impacted at each of the four velocities.

4.2 SPORES RELEASED FROM THE INTERIOR OF IMPACTED PELLETS

The percentage of spores released from the interior of the pellets after sand impact are presented in Table 3. The unreduced data is given in the Appendix.

Table 3: SPORES RELEASED FROM THE INTERIOR OF INTERNALLY CONTAMINATED PELLETS AFTER IMPACTION

Velocity m/sec	Mean Percent Spores Released (a) (10^4 Spores Available for Release) (b)
168	0.01
457	0.29
945	0.23
1554	0.34

(a) Mean of 6 Pellets

(b) Adjusted Spore Level

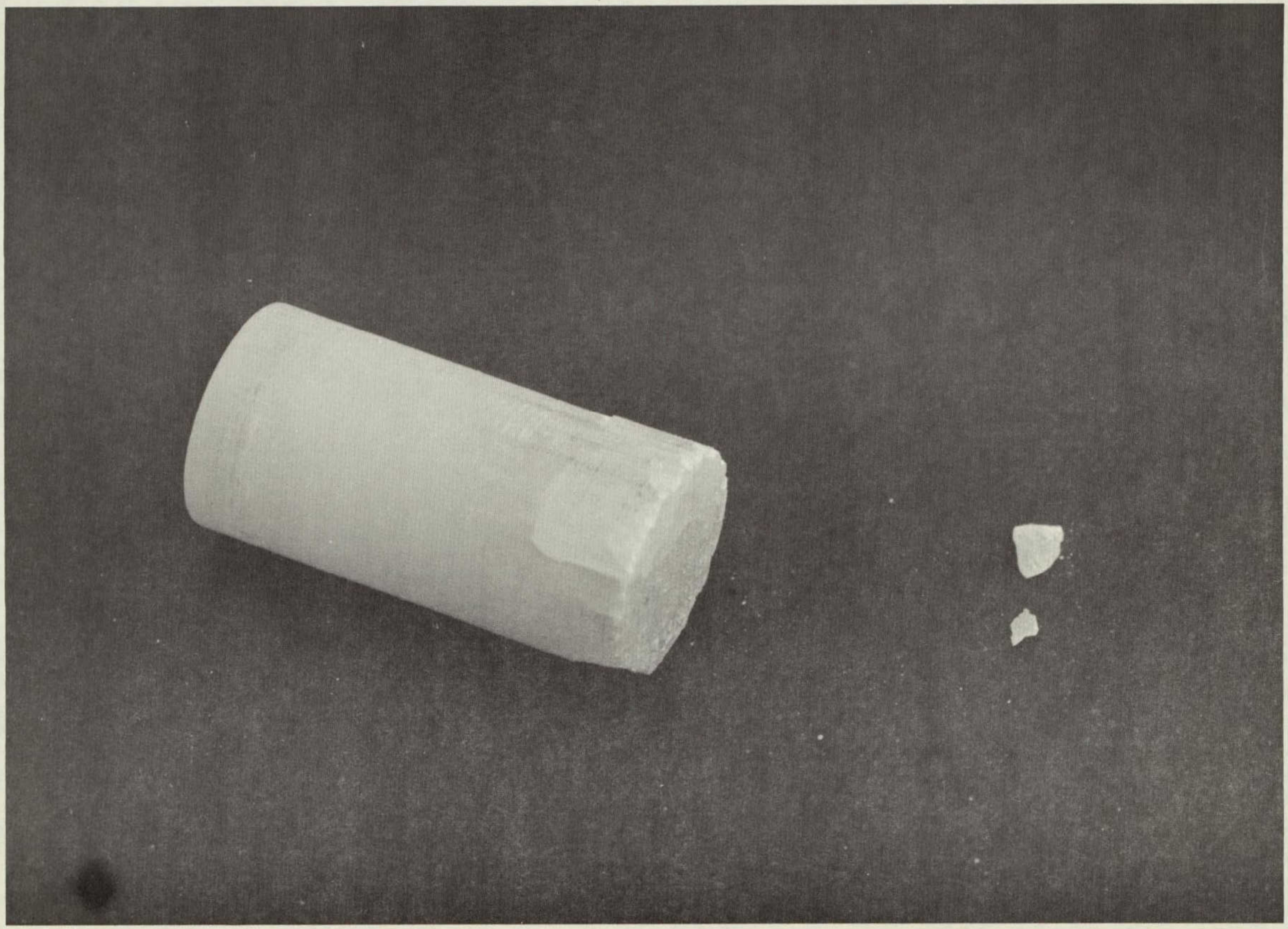


Figure 5: IMPACT AT 168 M/SEC

NOT REPRODUCIBLE

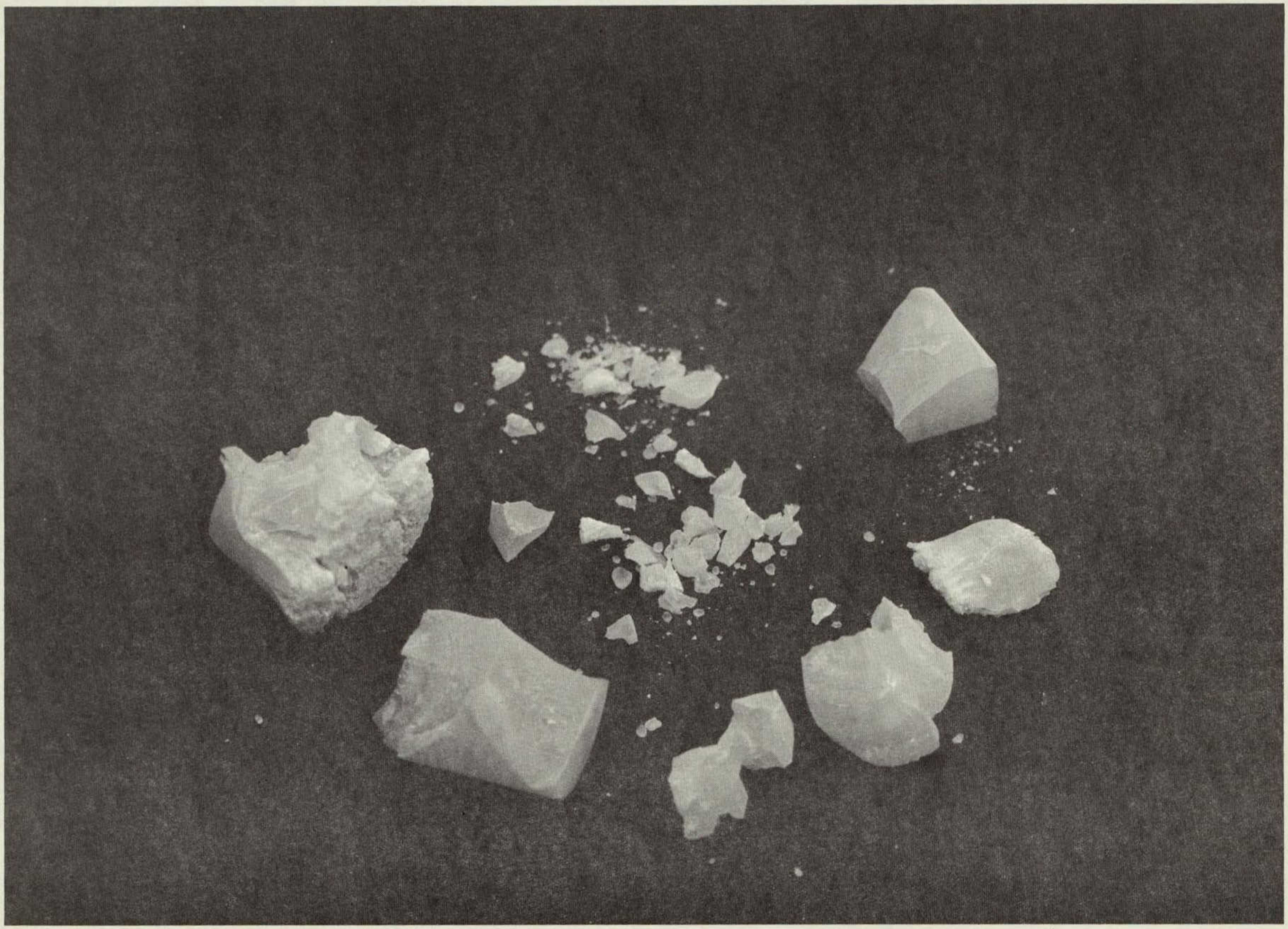
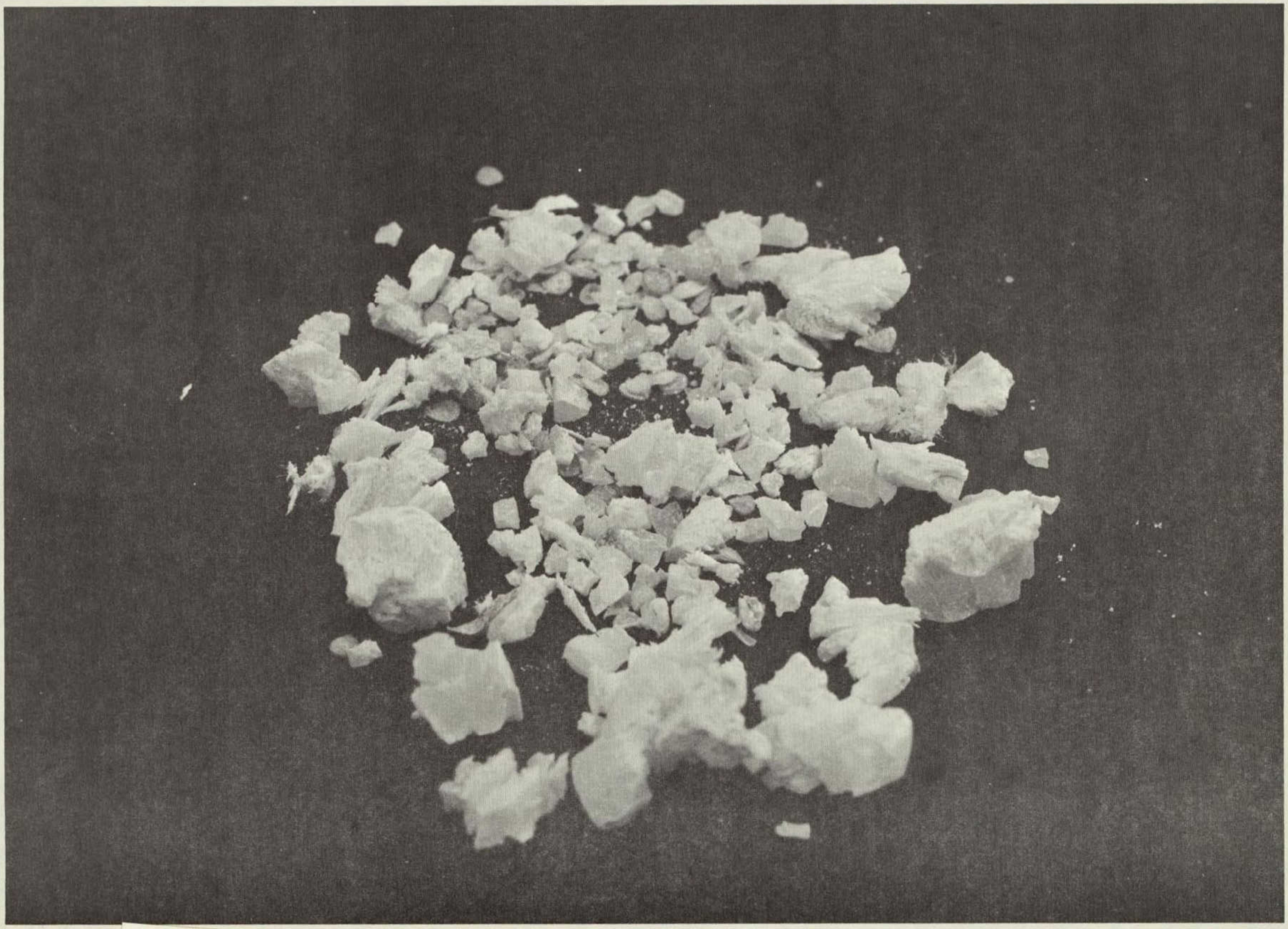


Figure 6: IMPACT AT 457 M/SEC

NOT REPRODUCIBLE



NOT REPRODUCIBLE

Figure 7: IMPACT AT 945 M/SEC

NOT REPRODUCIBLE

Figure 8: IMPACT AT 1,554 M/SEC

It was noted that, at the four test velocities, less than 1 percent of the available number of spores in the pellet were released.

4.3 TEST CONTROLS

No background contamination due to fabrication and handling of the material, or due to the recovery and analytical procedures was observed. In addition, the surface sterilization method was found to be effective for eliminating all viable external spores from the pellets.

4.4 STATISTICAL ANALYSIS

An applicable analysis of variance technique was employed to assess the data obtained on spore release. The results show a highly significant difference ($F = 10.59$ with 3 and 20 degrees of freedom) in the percentage of spores released at the four velocities. The differences were examined at the 1% level.

5.0 SUMMARY

This test was conducted to determine the number of microorganisms released from the interior of Eccobond pellets impacted onto sand. The pellets were fabricated so that each pellet interior contained approximately 1×10^4 *B. subtilis* var. *niger* spores. A series of pellets were propelled from a gun at 168, 457, 945 and 1554 m/sec. Impact occurred in sand which was contained in a collection canister. After impact, the pellet fragments and sand were analyzed to determine the number of viable spores released.

An analysis of variance of the percentage of spores released upon impact showed a significant difference between the four test velocities. However, less than 1 percent of the available organisms in the pellets was released upon impact at all test velocities.

6.0 REVISED HARD IMPACT DATA

A summary of the data from all hard impact studies conducted during the past three years are presented in this section. The microbial release data obtained during this Phase II study provided the additional information to supplement the hard impact release studies conducted previously. The grinding constant determined during Phase I has been applied to adjust the Eccobond data. Table 4 presents the revised data for the percent of microbial spores released upon impact. The log reductions in population from the initial spore levels after hard impact are given in Table 5.

As these summary data show, in no case were more than 1% of the total available spores released upon impact. Also, the log reductions from the initial spore levels were 2 or greater for all test conditions.

Table 4: PERCENT SPORES RELEASED AT IMPACT

	Methyl Methacrylate Onto Stainless Steel				Methyl Methacylate onto Sand	Eccobond * onto Stainless Steel	Eccobond * onto Sand
Initial Spore Level / Impact Velocity m/sec	10^2	10^3	10^4	10^5	10^4	10^4	10^4
168	0.6	0.1	0.2	0.03	0.001	0.31	0.01
457	0.4	0.6	0.6	0.3	0.001	0.98	0.29
945	0.6	0.8	0.1	0.02	0.06	0.64	0.23
1554	0.2	-	-	0.002	0.06	0.06	0.34

* Adjustment constant (20) applied.

Table 5: SPORES RELEASED AT IMPACT

LOG REDUCTIONS FROM INITIAL SPORE LEVEL

	Methyl Methacrylate onto Stainless Steel				Methyl Methacrylate onto Sand	Eccobond * onto Stainless Steel	Eccobond * onto Sand
Initial Spore Level / Impact Velocity m/sec	10^2	10^3	10^4	10^5	10^4	10^4	10^4
168	2.2	2.9	2.8	3.5	4.7	2.5	3.9
457	2.5	2.2	2.2	2.5	4.7	2.0	2.5
945	2.2	2.1	3.1	3.7	3.3	2.2	2.6
1554	2.7	-	-	4.7	3.3	3.3	2.5

* Adjustment constant (20) applied.

7.0 APPENDIX

The unreduced data for this investigation are given in Tables 6, 7, and 8.

Table 6: PERCENT PELLET RECOVERY OF ECCOBOND PELLETS FOLLOWING IMPACT ONTO SAND

Velocity m/sec	Pellet Number	Pellet Weight Prior to Launch, Grams	Recovered Pellet Weight After Impact, Grams	Percent, By Weight, Pellet Recovered
149	A-3	0.962	0.962	100.0
171	A-5	0.961	0.961	100.0
178	A-6	0.964	0.942	97.7
181	A-8	0.961	0.957	99.6
180	A-9	0.964	0.965	100.1
180	A-10	0.961	0.962	100.1
509	B-12	0.959	0.968	100.9
480	B-13	0.962	0.753	78.3
511	B-14	0.957	0.840	87.8
421	B-15	0.962	0.964	100.2
483	B-16	0.963	0.863	89.6
468	B-17	0.963	0.913	94.8
914	B-18	0.961	0.303	31.5
927	B-19	0.964	0.826	85.7
881	B-20	0.962	0.628	65.3
939	B-21	0.956	0.757	79.2
911	B-22	0.958	0.781	81.5
913	B-24	0.957	0.880	92.0
1606	B-25	0.962	0.449	46.7
1579	B-28	0.959	0.660	68.8
1591	C-37	0.958	0.775	80.9
1542	C-38	0.959	0.618	64.4
1582	C-39	0.957	0.758	79.2
1490	C-40	0.960	0.734	76.5

√

Table 7: TOTAL SPORE LEVEL OF ECCOBOND PELLETS

Pellet Number	Pellet Weight, Grams	Spore Count	Spore Count/ Gram Pellet	Total Spore Level/Gram Pellet (a)	Adjusted Total Spore Level/ Gram Pellet (b)
I-22	0.925	1450	1568	1445	28,900
I-23	0.878	1160	1321		
II-46	0.959	2393	2495	2202	44,040
II-47	0.950	1813	1908		
III-70	0.959	986	1028	1105	22,100
III-71	0.958	1131	1181		

(a) Mean for that plastic batch.

(b) Spore level increased by 20 times.

Table 8: SPORES RELEASED FROM INOCULATED ECCOBOND AFTER IMPACT ONTO SAND

Velocity m/sec	Pellet Number	Pellet Weight, Grams	Adjusted Initial Spore Level/G Pellet Before Impact	Number Spores Released Upon Impact	Number Spores/G Pellet Released Upon Impact	% Spores Released
183	I-1	0.958	28,900	6	6.3	0.02
146	I-2	0.960	28,900	1	1.0	0.003
177	I-4	0.956	28,900	5	5.2	0.02
182	I-5	0.957	28,900	5	5.2	0.02
166	I-6	0.953	28,900	0	0	0
176	I-7	0.960	28,900	5	5.2	0.02
495	I-8	0.950	28,900	24	25	0.09
434	I-9	0.956	28,900	70	73	0.25
466	I-10	0.952	28,900	152	160	0.55
494	I-11	0.955	28,900	81	85	0.29
451	I-12	0.959	28,900	120	125	0.43
469	I-13	0.959	28,900	40	42	0.15
884	II-24	0.960	44,040	127	132	0.30
860	II-25	0.958	44,040	195	204	0.46
924	II-26	0.958	44,040	75	78	0.18
881	II-28	0.959	44,040	53	55	0.12
838	II-29	0.957	44,040	54	56	0.13
960	II-31	0.958	44,040	87	91	0.21
1579	II-33	0.958	44,040	121	126	0.29
1533	II-38	0.954	44,040	167	175	0.40
1494	II-41	0.954	44,040	135	142	0.32
1554	III-53	0.963	22,100	78	81	0.37
1554	III-54	0.963	22,100	69	72	0.33
1544	III-55	0.964	22,100	74	77	0.35

SECTION IV

PHASE III

EROSION OF INOCULATED METHYL METHACRYLATE AND ECCOBOND BY SAND BLASTING

TABLE OF CONTENTS

PHASE III

EROSION OF INOCULATED METHYL METHACRYLATE AND ECCOBOND BY SAND BLASTING

1.0 PURPOSE

This study was conducted to determine the effect of the erosive action of sand on the release of bacterial spores from methyl methacrylate and Eccobond.

2.0 INTRODUCTION

Methyl methacrylate and Eccobond have been impacted at various velocities onto stainless steel and sand. The results from these studies show that viable spores remain in the fractured material. Even though all of these spores were not released by impact they are still available for release by erosion. Therefore, this program was initiated to study the effect of aeolian erosion on the release of encapsulated microorganisms.

3.0 PROCEDURE

Methyl methacrylate and Eccobond discs were fabricated so that each disc contained approximately 4×10^4 *Bacillus subtilis* var. *niger* spores. A disc was placed in a specially designed sand blasting device which utilized the Venturi principle to accelerate sand which was directed towards the disc. Tests were conducted using both filtered air and carbon dioxide to accelerate the sand. The carbon dioxide tests were conducted to determine if the carrier gas had an effect on microbial survival and to more closely approximate Martian atmospheric conditions. Three exposure times of 0.5, 2 and 24 hours were used for the air tests. One exposure time of 0.5 hours was used for carbon dioxide. After sand blasting, the remaining disc, sand, dust and filter were analyzed for viable spores that had been released by erosion. The erosion tests are outlined in Table 1.

3.1 DISC MANUFACTURE

3.1.1 Preparation of Spore Stock

A 0.1 ml aqueous suspension containing 1×10^8 *B. subtilis* spores was placed in the bottom of sterile planchets. The stock spore suspension was

Table 1: PHASE III - EROSION TESTS

Environment	Exposure Time	Methyl Methacrylate		Eccobond	
		Released Count after Erosion 10,000 spores/gm	Procedural Controls (unseeded)	Released Count after Erosion 10,000 spores/gm	Procedural Controls (unseeded)
Air	30 min.	6*	1	6	1
	120 min.	6	1	6	1
	24 hr.	6	1	6	1
CO_2	30 min.	6	1	6	1
Total		24	4	24	4

Total: 56 Discs

* Number of Replicate Discs

a minimum of 6 months old. The planchets were dried overnight by airflow from a Class 100 clean bench. When all the water had evaporated, 10 planchets were placed in a bottle containing 30 ml of ethanol and insonated for 20 minutes. The level of the bath water was adjusted so that it was halfway up the side of the bottle. After insonation, the 30 ml spore-ethyl alcohol suspension was pipetted into a sterile, capped bottle. All planchets were rinsed in fresh ethanol and the washings plus additional ethanol were combined with the 30 ml stock to bring the total to 100 ml. The stock inoculum was refrigerated until used. A plate count of the spore stock was performed 1, 3 and 7 days after preparation to accurately determine the number of spores per ml.

3.1.2 Inoculation of Methyl Methacrylate Powder

One hundred grams of methyl methacrylate powder were placed in a sterile 150 mm petri plate. The desired number of spores plus enough ethyl alcohol to bring the volume to a total 100 ml was throughly mixed with the powder. The wet seeded powder was air dried in a Class 100 clean bench overnight. The dried seeded powder was sifted through a 40 mesh screen and stored in a glass beaker. All seeded powder was used within one week of preparation.

3.1.3 Removal of Preservative From Liquid Methyl Methacrylate

One hundred ml of liquid methyl methacrylate were placed in a 250 ml separatory funnel. A 100 ml freshly prepared 2% solution of sodium hydroxide was then added. The mixture was gently swirled for one minute and allowed to separate into two fractions. The bottom pink fraction containing the preservative was discarded. Another 100 ml of 2% sodium hydroxide was added, and the washing process repeated until no pink color was observed in the bottom layer. When the bottom layer was clear, one additional washing with 100 ml sodium hydroxide was performed. The washed liquid methyl methacrylate was rinsed with separate 100 ml volumes of distilled water to remove all traces of sodium hydroxide. The number of rinses was one more than the sodium hydroxide. All water (bottom layer) was drained from the funnel. The washed liquid methyl methacrylate was used within 24 hours of preparation.

3.1.4 Polymerization of Methyl Methacrylate

Forty ml of washed liquid methyl methacrylate were added to 40 gm of seeded powder in a glass beaker and mixed until a uniform slurry was obtained. The mixture was poured into glass test tubes (13 x 100 mm) so that they were 2/3 full. As each tube was filled, the beaker was swirled to assure a homogeneous mixture. When all tubes were filled, they were immediately placed in a desiccator jar and the pressure reduced to 127 mm of mercury for 10 minutes. This vacuum is sufficient to create a slow bubbling action of the mixture in the tubes, but not so low as to cause the mixture to "climb" out the tube as the air is removed by the vacuum. The tubes were then transferred for curing to a 50°C water bath and heated for 1.5 hours. The water level of the bath was adjusted so that it was slightly above the plastic level in the tubes. After 1.5 hours the tubes were removed from the bath and allowed to cool at room temperature for 10 minutes. The fabricated plastic rods were removed by breaking the glass tube. The rods were then stored in a glass jar in the freezer (-18°C) until they were machined into pellets.

3.1.5 Inoculation and Polymerization of Eccobond

Thirty-six grams of Eccobond 55 were placed in a glass beaker and 4.32 grams of Catalyst 9 added. The required number of spores in 0.2 ml of alcohol were added and the mixture thoroughly stirred. The glass beaker containing the liquid Eccobond was placed in a desiccator jar and the pressure reduced to 127 mm of mercury for 10 minutes. The mixture was then removed from the jar and poured into 13 x 100 mm Teflon tubes. The tubes were cured in a 50°C oven for 3 hours.

After 3 hours, the tubes were removed from the oven, allowed to cool at room temperature for 10 minutes, and the Eccobond removed from the tubes. The Eccobond rods were stored in a glass jar in the freezer (-18°C) until machining.

3.1.6 Disc Fabrication

Seeded methyl methacrylate and Eccobond rods were machined into discs by turning the rods on a lathe. The machining sequence was to cut all rods from one batch of plastic to an established diameter. Face cuts were

then made to finish the discs to the correct length. Each finished disc was 1.27 cm in diameter, 0.71 cm long and weighed approximately 1 gm.

3.1.7 Disc Surface Sterilization

After machining, each disc was numbered, weighed to the nearest one-thousandth of a gram and this information recorded. The discs were surface sterilized in a freshly prepared 2000 ppm chlorine solution for 10 minutes. This was followed by a 10 minute soak in a fresh filter-sterilized 2% solution of sodium thiosulfate. Then, the discs were exposed to the airflow of a Class 100 clean bench for 15 minutes to evaporate any moisture on the surface. Each disc was stored in a freezer in a sterile, appropriately labelled, screw-capped test tube until used.

3.2 SAND BLASTING APPARATUS

The simulation of aeolian erosion was accomplished by utilizing the Venturi principle in a specially designed sand blasting device (Figure 1). A controlled flow of filtered air or carbon dioxide was metered (Rotameter, Brooks, Instrument Co.) into the apparatus. The pressure differential created by the Venturi arrangement picked sand up from the reservoir and the gas flow through the nozzle accelerated the sand which was directed towards the target. The design of the apparatus allowed the sand to drop back down into the reservoir for recirculation. Any dust that was formed was carried with the gas flow and trapped in the cyclone separator. Very fine dust particles not trapped in the separator were carried out and trapped on a 0.45 μ membrane filter.

3.3 EROSION TESTS

The sand blasting apparatus was sterilized in a hot air oven at 150°C for 24 hours. Eighty grams of sterilized sand (washed and ignited sand, J. T. Baker Co.) were placed in the sand reservoir. A disc was attached to the target holder with sterile double-backed tape and a sterile filter was placed in the filter holder. The apparatus was then assembled and connected to either the air or carbon dioxide supply and the proper flow adjustments made with the rotameter. Figure 2 is a photograph of the test set-up. The rate of erosion was varied for each of the four test conditions (i.e., air tests of 0.5, 2 and 24 hours and a CO_2 test of 0.5 hours) so as to erode

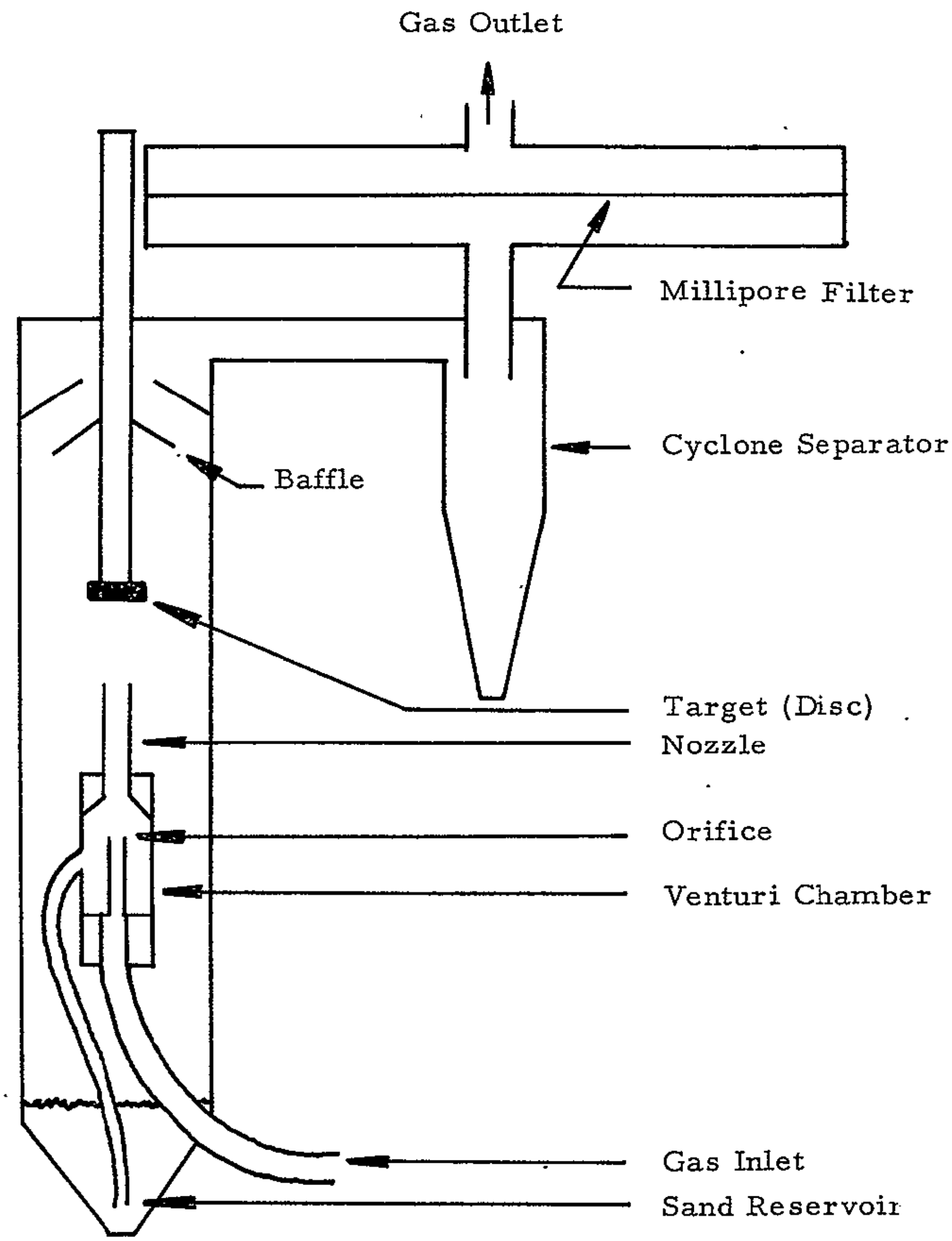


Figure 1: SCHEMATIC OF SAND BLASTER

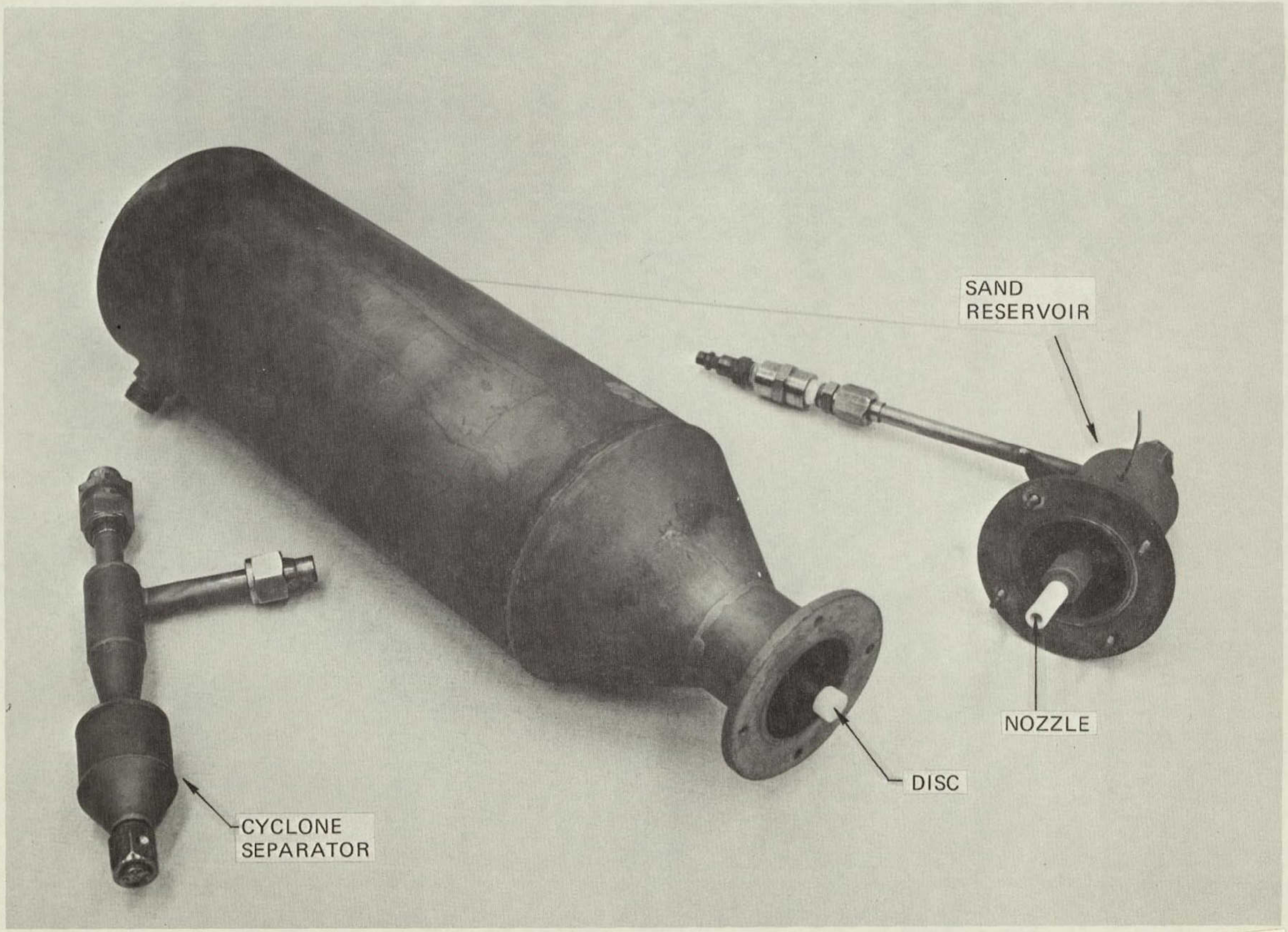


Figure 2: SANDBLASTING APPARATUS

NOT REPRODUCIBLE

0.25 ± 0.05 grams from each test disc. The amount of erosion was determined by weighing each disc before and after sand blasting. The assembly and disassembly of the apparatus was performed in a Class 100 clean bench.

3.4 SPORE RECOVERY ANALYSIS

After sand blasting, the apparatus was disassembled for analysis of the contents, Figure 3. The remaining sand in the reservoir was removed and distributed into petri dishes and covered with Trypticase Soy Agar (TSA). The dust that had collected in the cyclone separator was removed and also distributed into petri dishes and mixed with TSA. The 0.45 μ filter was removed from the holder and overlayed with TSA in a petri dish. The apparatus was then rinsed twice with 500 ml aliquots of sterile distilled water which was filtered through several membranes. These filters were then placed on TSA in a petri dish. After rinsing, the disc was removed and weighed. For the 6 replicate discs eroded for each test condition, 3 of these discs were placed in petri dishes and overlayed with TSA. The other 3 discs were assayed to obtain the number of viable spores remaining in each disc. These assay procedures are given in the following paragraph. All of the agar plates were incubated for 2 weeks at 30°C. Each colony counted on the plates was recorded as one spore released from the disc.

3.5 ENUMERATION OF SPORES IN DISCS

Acetone was used as the solvent for dissolving methyl methacrylate. Four hundred ml of acetone were used to dissolve 1 gram of methyl methacrylate. The acetone and methyl methacrylate were contained in a one liter, screw-capped bottle and placed on a refrigerated reciprocal shaker maintained at 10°C. Approximately 24 hours were required for complete dissolution. After dissolution, one ml aliquots were removed from the bottle and filtered through 0.5 μ membranes. Each filter was placed on TSA and incubated for 72 hours at 30°C.

A Waring blender was modified for grinding Eccobond discs, Figure 4. The cutting blades were replaced with a flat metal surface to firmly hold an aluminum oxide paper disc (Rub Wet, 240 grit, Armour Star Co.). The

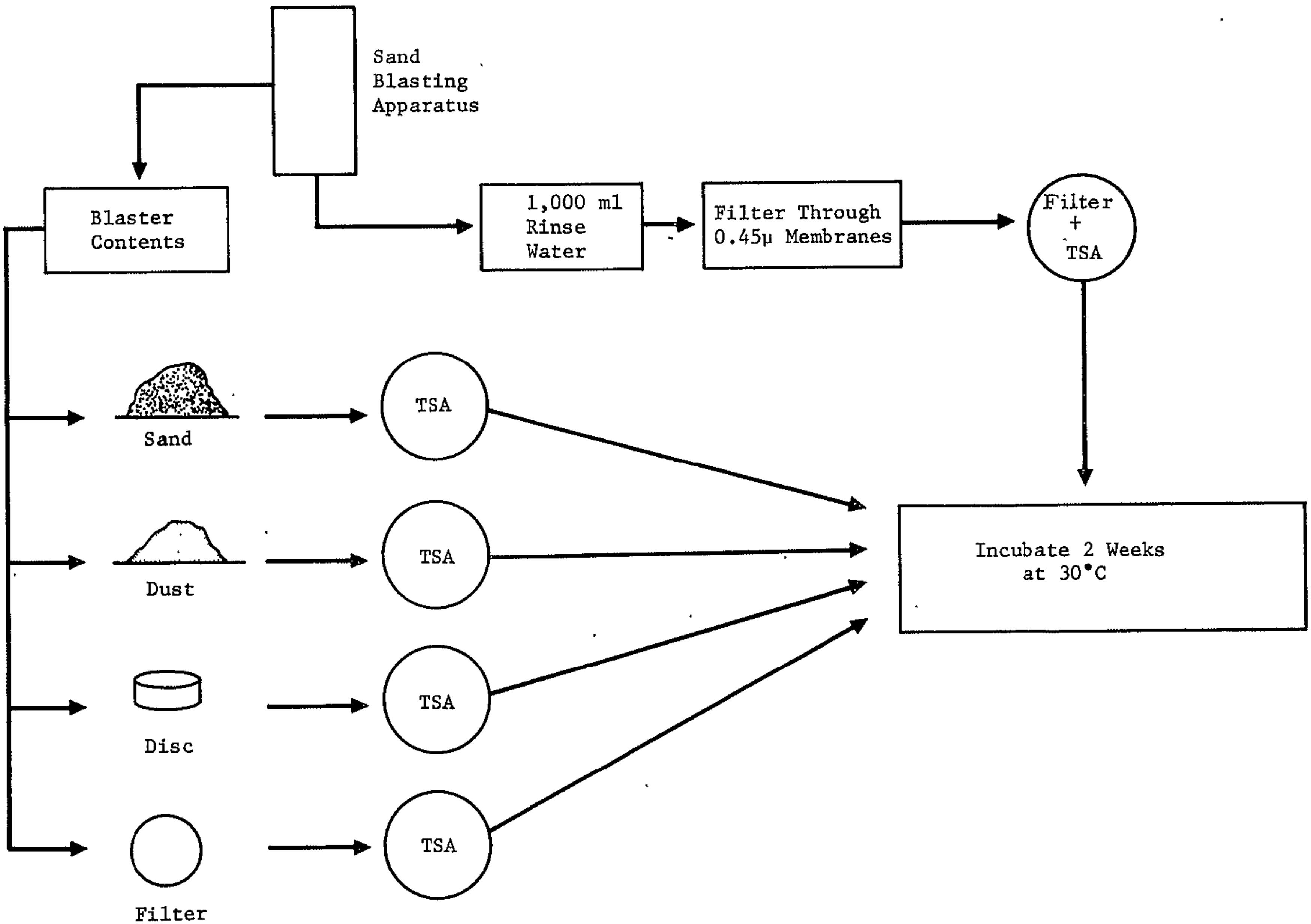


Figure 3: RELEASED SPORE ANALYSIS

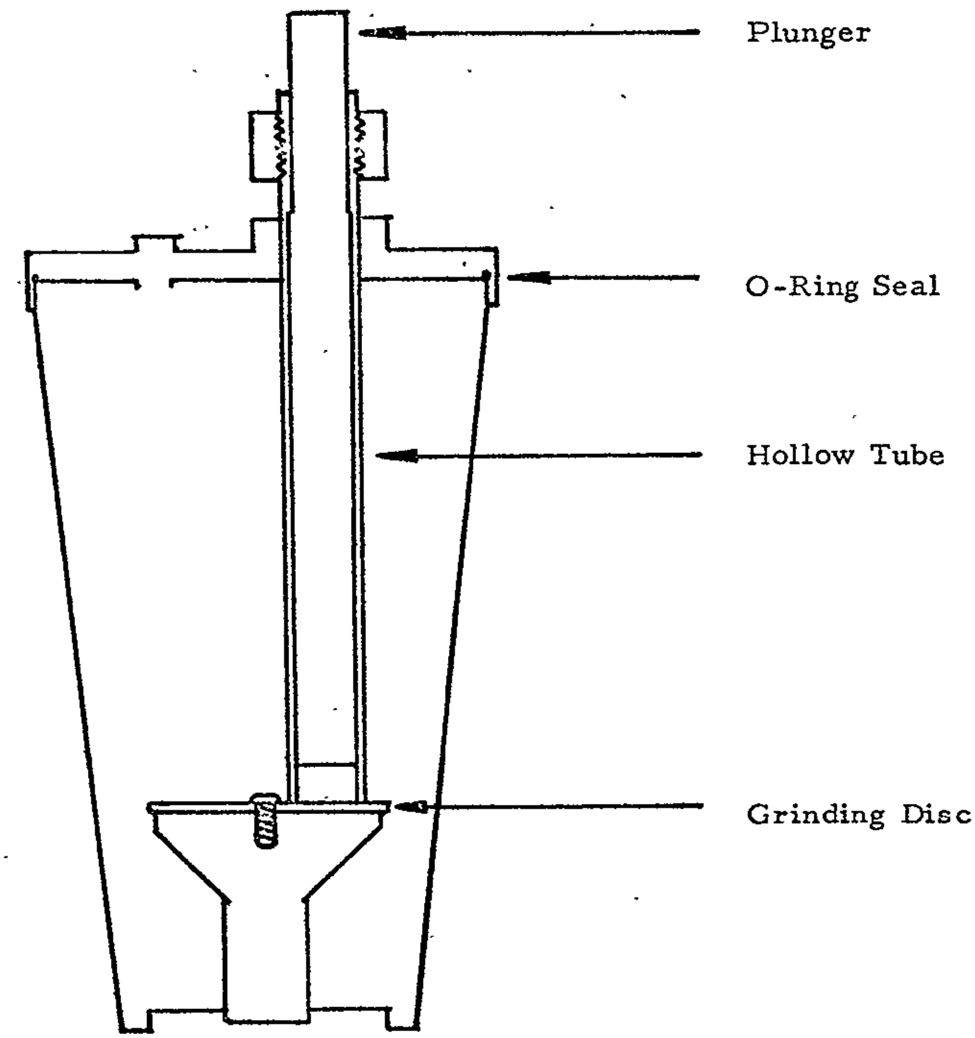


Figure 4 : MODIFIED WARING BLENDER

cover of the blender jar was modified by drilling a hole through it and inserting a tube down through the blender jar so as to just clear the top of the abrasive disc. A second tube, fitted with a Teflon plug, was used as a push rod to hold a disc firmly against the abrasive paper.

Following assembly of the blender, 150 ml of sterile distilled were added to it, the blender placed on the motor and activated for 1 minute. A 5 ml aliquot of water was then removed and plated in TSA to serve as a sterility control.

After removing the push rod, a disc was dropped into the tube and the push rod reinserted. The blender was placed on the motor, the motor activated and a slight pressure was exerted on the push rod to hold the disc against the grinding disc. Approximately 10 seconds were required to grind a disc. Ten ml aliquots of the suspension were plated in TSA and incubated for 72 hours at 30°C.

3.6 PROCEDURAL CONTROLS

Unseeded, sterile discs were exposed to the abrasive action of sand in the erosion apparatus. An analysis of the erosion products was conducted in the same manner as described in paragraph 3.4. These controls were used to establish the reliability of the test data with respect to possible contamination due to procedural techniques.

3.7 TEMPERATURE AND HUMIDITY MEASUREMENTS

Temperature changes occurring from sand impaction on the discs were measured. Thermocouples were embedded near the surface of the discs and each disc was eroded to the point of breaking the thermocouple. Temperatures were measured with a pyrotest thermocouple potentiometer.

The humidity of the air and CO_2 was measured with a dew point recorder. The gases were measured at both the nozzle and outlet portion of the sand blasting apparatus.

4.0 RESULTS AND DISCUSSION

4.1 EROSION RATES

The gas flow metered into the sand blasting apparatus was varied with each test condition to effectively erode 0.25 $\pm$ 0.05 gm from each disc. Table 2 gives the parameters used for each test condition. Figure 5 shows a methyl methacrylate and Eccobond disc with approximately 0.25 grams eroded.

Table 2: TEST PARAMETERS

Test Condition	I. D. Orifice mm	I. D. Nozzle mm	Flow Rate lpm
0.5 hour - CO_2	2.36	4.70	105.2
0.5 Hour - Air	2.44	4.70	134.4
2 Hour - Air	2.44	4.70	119.3
24 Hour - Air	2.36	4.70	66.2

4.2 SPORES RELEASED BY EROSION

A summary of the spores released by aeolian erosion is presented in Table 3. The unreduced data for these summary figures are given in the Appendix. It was noted that the average percent of spores released based on the number of spores available for release is less than 1 percent for all test conditions.

An appropriate analysis of variance technique was used to assess the data obtained on spore releases. The results of this analysis show no significant differences ($F = 1.42$ with 7 and 40 degrees of freedom) between the 4 test conditions or between the 2 materials.

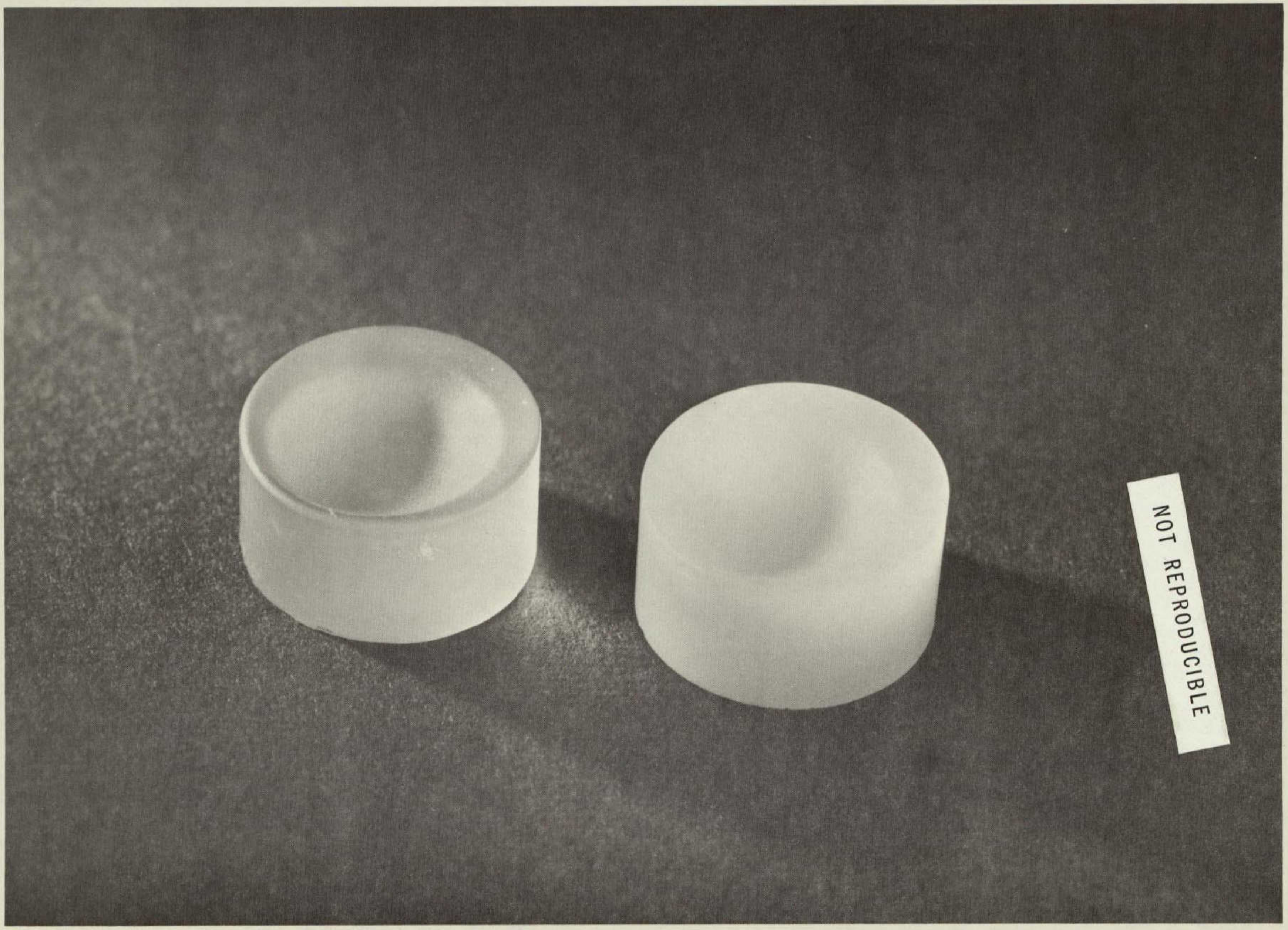


Figure 5: ERODED DISCS

NOT REPRODUCIBLE

Table 3: SUMMARY OF RELEASED SPORES BY AEOLIAN EROSION

METHYL METHACRYLATE 34,800 Average Spores per Gram Material [1]				
Exposure	Average Grams Eroded	Average No. Spores Available in Eroded Material	Average No. Spores Released	Average % of Available Spores Released
CO_2 - 0.5 hr	.2260 [2]	7900	8.8	0.11
Air - 0.5 hr	.2196	7600	1.8	0.02
Air - 2 hrs	.2321	8100	0.8	0.01
Air - 24 hrs	.2432	8500	1.5	0.02

ECCOBOND 38,600 Average Spores per Gram Material [1]				
Exposure	Average Grams Eroded	Average No. Spores Available in Eroded Materials	Average No. Spores Released	Average % of Available Spores Released
CO_2 - 0.5 hr	.2701 [2]	10400	3.0	0.03
Air - 0.5 hr	.2354	9100	0.2	0.002
Air - 2 hrs	.2555	9900	1.5	0.02
Air - 24 hrs	.2418	9300	1.3	0.01

1 Average based on 12 replicates.

2 Average based on 6 replicates.

4.3 SPORES REMAINING IN ERODED DISCS

One-half of the eroded discs were assayed to determine the number of viable spores remaining in the disc. A summary of these data are presented in Table 4. See Appendix for raw data.

Table 4: VIABLE SPORES IN DISC AFTER EROSION

Exposure	Methyl Methacrylate Average Number Spores Remaining in Disc	Eccobond Average Number Spores Remaining in Disc
CO_2 - 0.5 hours	28,400*	36,900*
Air - 0.5 hours	31,200	32,800
Air - 2 hours	30,400	32,400
Air - 24 hours	22,700	33,000

* Average based on 3 replicates.

4.4 PROCEDURAL CONTROLS

Uninoculated, sterile discs were exposed to the same erosion conditions as the test discs. The results of the microbial analysis of these discs showed no recovery of B. subtilis spores.

4.5 TEMPERATURE AND HUMIDITY

The results of the temperature measurements taken during the erosion process are shown in Table 5. These figures represent the rise in temperature above ambient which was measured as 23°C. No differences were detected between methyl methacrylate and Eccobond discs. The results of the humidity measurements are given in Table 6.

Table 5: EROSION TEMPERATURES

Exposure	Temperature Rise ^{o}C Above Ambient
CO_2 - 0.5 hours	0
Air - 0.5 hours	8
Air - 2 hours	7
Air - 24 hours	1

Table 6: RELATIVE HUMIDITY

Exposure	% Relative Humidity Gas Inlet at Nozzle	% Relative Humidity Gas Outlet at Filter
CO_2 - 0.5 hour	4.0	5 min = 3.5 25 min = 0.8
Air - 0.5 hour	22.4	5 min = 6.6 25 min = 5.4
Air - 2 hour	21.7	5 min = 5.9 60 min = 8.0 115 min = 7.3
Air - 24 hour	17.0	1 hr. = 4.6 7 hrs = 7.6 23 hrs = 9.5

5.0 SUMMARY

This study was conducted to determine the effect of aeolian erosion on the release of microorganisms from solid materials. Methyl methacrylate and Eccobond discs were fabricated so that each disc contained approximately 4×10^4 *B. subtilis* var. *niger* spores. The inoculated discs were eroded with sand in a specially designed sand blasting apparatus. Erosion rates were varied to erode 0.25 ± 0.05 gm of material from each disc for exposure tests of 0.5 hours using carbon dioxide, and 0.5, 2 and 24 hours using air. Following erosion, the sand, dust, disc and filter were assayed for released spores.

An analysis of variance of the percentage of spores released showed no significant difference between exposure tests or between the materials. Less than 1 percent of the available spores was released by erosion in all cases.

6.0 APPENDIX

The raw data for this program are presented in Tables 7 through 12.

Table 7: INITIAL NUMBER OF SPORES PER ONE GRAM DISC

Replicates	Methyl Methacrylate	Eccobond
1	32,109	30,500
2	35,711	46,460
3	32,586	33,560
4	30,325	34,120
5	30,811	43,960
6	38,724	40,540
7	35,616	47,160
8	35,171	34,340
9	34,735	33,000
10	37,839	35,420
11	34,490	43,300
12	39,321	40,900
Mean	34,800	38,600

Table 8: 0.5 HOURS EROSION WITH CARBON DIOXIDE

Replicates	METHYL METHACRYLATE			
	Grams Eroded	Number Spores Available in Eroded Material	Number Spores Released	% of Available Spores Released
1	.2320	8074	4	0.050
2	.2429	8453	41	0.485
3	.2239	7792	3	0.039
4	.2068	7197	3	0.042
5	.2347	8168	0	0
6	.2156	7503	2	0.027
Mean	.2260	7900	8.8	0.11

Replicates	ECCOBOND			
	Grams Eroded	Number of Spores Available in Eroded Material	Number Spores Released	% of Available Spores Released
1	.2954	11402	4	0.035
2	.2540	9804	5	0.051
3	.2879	11113	2	0.018
4	.2622	10121	0	0
5	.2699	10418	3	0.029
6	.2514	9704	4	0.041
Mean	.2701	10400	3.0	0.03

Table 9: 0.5 HOURS EROSION WITH AIR

Replicates	METHYL METHACRYLATE			
	Grams Eroded	Number Spores Available in Eroded Material	Number Spores Released	% Available Spores Released
1	.2059	7165	1	0.014
2	.2195	7639	1	0.013
3	.2145	7465	0	0
4	.2115	7360	7	0.095
5	.2245	7813	2	0.026
6	.2416	8408	0	0
Mean	.2196	7600	1.8	0.02

Replicates	ECCOBOND			
	Grams Eroded	Number Spores Available, in Eroded Material	Number Spores Released	% of Available Spores Released
1	.2086	8052	0	0
2	.2113	8156	1	0.012
3	.2416	9326	0	0
4	.2698	10414	0	0
5	.2638	10183	0	0
6	.2171	8380	0	0
Mean	.2354	9100	0.2	0.002

Table 10: 2 HOURS EROSION WITH AIR

Replicates	METHYL METHACRYLATE			
	Grams Eroded	Number Spores Available in Eroded Material	Number Spores Released	% of Available Spores Released
1	.2561	8912	0	0
2	.2139	7444	0	0
3	.2530	8804	1	0.011
4	.2245	7813	0	0
5	.2021	7033	0	0
6	.2430	8456	4	0.047
Mean	.2321	8100	0.8	0.01

Replicates	ECCOBOND			
	Grams Eroded	Number Spores Available in Eroded Material	Number Spores Released	% of Available Spores Released
1	.2593	10009	2	0.020
2	.2950	11387	1	0.009
3	.2349	9067	0	0
4	.2662	10275	3	0.029
5	.2428	9372	1	0.011
6	.2349	9067	2	0.022
Mean	.2555	9900	1.5	0.02

Table 11: 24 HOURS EROSION WITH AIR

Replicates	METHYL METHACRYLATE			
	Grams Eroded	Number Spores Available in Eroded Material	Number Spores Released	% of Available Spores Released
1	.2303	8014	2	0.025
2	.2341	8147	1	0.012
3	.2238	7788	0	0
4	.2618	9111	1	0.011
5	.2556	8895	5	0.056
6	.2536	8825	0	0
Mean	.2432	8500	1.5	0.02

Replicates	ECCOBOND			
	Grams Eroded	Number Spores Available in Eroded Material	Number Spores Released	% of Available Spores Released
1	.2160	8338	4	0.048
2	.2446	9442	0	0
3	.2575	9940	1	0.010
4	.2344	9048	1	0.011
5	.2383	9198	0	0
6	.2599	10032	2	0.020
Mean	.2418	9300	1.3	0.01

Table 12: NUMBER OF VIABLE SPORES REMAINING IN DISC AFTER EROSION

Exposure	Replicates	Methyl Methacrylate	Eccobond
CO_2 - 0.5 hours	1	30,251	42,780
	2	26,995	37,440
	3	27,863	30,580
Air - 0.5 hours	1	33,829	30,880
	2	28,168	41,100
	3	31,475	26,300
Air - 2 hours	1	27,689	31,000
	2	32,829	35,860
	3	30,743	30,400
Air - 24 hours	1	24,733	36,300
	2	20,159	37,820
	3	23,335	24,800